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CONTENTS

PAUL E. STEINER, D. WARREN STANGER, and MIRIAM N. BOLYARD. Comparison of the Carcinogenic Activity in Extracts of Human Liver and Other Human and Animal Organs 273

JOHANNES CLEMMESEN, and THOGER BUSK. Cancer Mortality among Males and Females in Denmark, England and Switzerland. I. 281

JOHANNES CLEMMESEN, and THOGER BUSK. Cancer Mortality among Males and Females in Denmark, England and Switzerland. II. Danish Towns and Rural Areas 286

HUGH J. CREECH, EVELYN L. OGINSKY, and F. S. CHEEVER. Immunological Studies of Hydrocarbon-Protein Conjugates. I. Precipitin Reactions 290

HUGH J. CREECH, EVELYN L. OGINSKY, and O. N. ALLEN. Immunological Studies of Hydrocarbon-Protein Conjugates. II. Quantitative Results 297

HUGH J. CREECH. EVELYN L. OGINSKY. and MAX TRYON. Immunological Studies of Hydrocarbon-Protein Conjugates. III. Inhibition Reactions 301

J. G. MCDONALD. Production of Reticulum Cell Sarcoma and Fibrosarcoma by Methylcholanthrene Adsorbed on Activated Carbon..... 305

L. A. STRAIT, and K. B. DEOME. Desoxycorticosterone Acetate, Mammary Gland Growth, and Carcinogenesis in Mice 310

ABSTRACTS 312-318
Reports of Research 312-314
Clinical and Pathological Reports 314-318

BOOK REVIEWS 319-320

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THE INDUCTION OF TUMORS

III. Extrinsic Factors

IV. Intrinisic Factors

ATTEMPTS AT CONTROL OF TUMOR INDUCTION AND OF TUMOR GROWTH

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CANCER RESEARCH
A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH
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Comparison of the Carcinogenic Activity in Extracts of Human Liver and Other Human and Animal Organs*

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(Received for publication November 25, 1946)

INTRODUCTION

Evidence has been slowly accumulating for many years that certain exogenous substances occasionally induce cancer in man (9). The principle has been established that some human neoplasms may be caused by exogenous chemicals. It remains to be determined how completely the etiology of human tumors is explained by an extension of this principle. During the past 14 years a great number of chemicals were shown to be carcinogenic in experimental animals (5). The chemical nature of some of these was such as to suggest the possibility that related endogenous carcinogens might exist in man and perhaps be responsible for some of his visceral neoplasms. The logical next step was to examine human tissues, secretions, and excretions for substances, exogenous or endogenous, having tumor-inducing activity. Such studies were begun in a number of laboratories during the past decade. They have produced evidence that carcinogenic factors exist in extracts of human materials. To date these carcinogens have not been identified and their origin and relation, if any, to human tumors is not known, but the findings provide a fertile field for investigation and speculation. In this paper reference is made only to tissue extracts; those from secretions and excretions are omitted because they were recently reviewed (24).

Tumor-inducing activity has been demonstrated in extracts of non-cancerous livers from persons who had cancer, by Schabad (17), Kleinenberg, Neufach, and Schabad (10, 11), Des Ligneris (3), Hieger(6), Steiner (20, 22, 25), Tanaka (29), Cracium, Zugravescu, Ursu and Manulescu-Motoc (2), Sannié, Truhaut and Guérin (16). Similar activity has been found in extracts of liver from persons who had no cancer by Kleinenberg and his associates (10) and Steiner (22, 25). Des Ligneris (3) reported that extracts of livers from Bantus who had no tumor were carcinogenic but those from Europeans were not. Hieger (6) also demonstrated tumorigenic activity in noncancerous Bantu livers, but Steiner (25) found the factor in the American Negro to have the same incidence as in whites. Only Gummel (4) reported failure to find carcinogenic activity in extracts from livers.

Both carcinomas and sarcomas have been induced by liver extracts. Local sarcogenic activity has been demonstrated by subcutaneous injection (6, 10, 11, 16, 17, 20, 22, 25, 29), and carcinogenicity was shown by epidermoid carcinomas which followed skin painting (3), and by adenocarcinomas, which were thought to have been induced from mammary gland, at the point of subcutaneous injections (10, 11, 17).

The neoplasms developing at the point of application of the extracts have generally been accepted as having been induced. Tumors were considered to have been induced at distant sites by some authors (10,11,16) but not by others (22,25).

The tumor-inducing factor was found in the total lipids extractable with benzene (10,11,17), and in the nonsaponifiable lipid fraction extracted with ethylene dichloride (20,22,25) or with petroleum ether (3,6,16). The percentage of nonsaponifiable residue found in 248 cancer livers was 0.681 and in noncancer livers, 0.657 (18). About half of this material was shown to be cholesterol.

In experiments with extracts from 67 livers, 14 had demonstrable tumor-producing activity, 13.1 per cent of the mice developing sarcoma (25). No relationship of activity was observed as to type or site of the primary tumor, or to the type of major dis-

* This work was aided by a grant from the Commonwealth Fund.

ease, except that 4 cases had a disturbance of the endocrine glands concerned with steroid hormones. Neither was there any recognized relationship to race, sex, age, or cirrhosis in the liver.

Extracts of cancer tissue have also been tested. Menke reported positive results with human mammary gland cancers (13,14) and with human osteogenic sarcoma. Des Ligneris (3) found evidence of activity in extracts of the primary carcinomas in Bantu livers from which the non-neoplastic liver tissue had been separated. Steiner (22) was unable to find activity in extracts of large amounts of pooled cancer tissue.

Other materials of human origin that have been tested have recently been reviewed (24). To them should be added the observations of Hirschmann, Heinle and Wearn (8) on a urinary fraction that produces myeloid metaplasia, and of Turner and Miller on the induction of lymphoid hyperplasias or with extracts from human leukemic urine and feces (15) and bovine liver (30).

Tissues from animals that have been tested and reported positive include lactating mammary glands from DBL strain mice (7), rat sarcomas and rat livers from sarcoma patients (1), and pituitary glands (anterior and posterior lobes) of cattle (31).

The liver on theoretical grounds gave greater promise of containing exogenous or endogenous carcinogens than any other tissue in the body, except possibly for tumors themselves or for precancerous sites or lesions (precancerous not in the histological sense but on the basis of probability of subsequently undergoing neoplastic transformation in the light of past experience with similar cases). Liver was used because of: (a) Its role in the metabolism of sex hormones, bile acids, cholesterol and other sterols chemically related to some of the potent synthetic carcinogens. (b) Its function as a great detoxifier and excretor of many substances. Perhaps exogenous carcinogens, particularly those absorbed from the alimentary tract, would be taken there for inactivation or excretion. (c) Its availability in great bulk as a possible precancerous lesion in cases of cirrhosis (3), in which a hypothetical carcinogen might be responsible for causing both diseases. (d) The incidence of carcinoma of the extrahapetic bile ducts which, although it is not among the commonest neoplasms, occurs often enough to suggest that extraordinary carcinogenic influences are being exerted on these epithelial cells, of which, considered as individuals, there are so few available for neoplastic transformation (22). (e) Its high lipid content, which might act as solvent for exogenous or endogenous carcinogens. (f) Its availability in large quantities for chemical extraction and biological testing.

After the demonstration that liver contains carcinogenic substances, it became of interest to know whether this is a property of liver only or of other organs and tissues as well, or perhaps even of all protoplasm. To date, reports are available on human adrenal glands and hyperplastic prostates which gave negative results (6), and on human lungs for which there is some positive evidence, although slight (11). If the body tissues in general were to give negative results the possibility that the carcinogen in some livers is a chemical conversion product would be lessened and the theoretical considerations given above might be strengthened. Accordingly experiments were begun with many kinds of organs, tissues, and other materials from human and animal sources in a survey of the body. Some of these experiments are reported in this paper and others will be presented later.

Extracts were prepared from livers, kidneys, spleens, hearts and colons of persons who died of malignant tumors. The tissues used for extraction contained no cancerous tissue but for brevity they are hereafter called "cancer" extracts. A corresponding series of tissues from noncancer patients was simultaneously tested and these are hereafter referred to as "noncancer" extracts. An extract of pooled livers from stillborn infants was also tested. As these infants had taken no food by mouth from the external environment, it was thought that if their livers contained carcinogenic activity, this would be evidence for the endogenous nature of the substance. It was also deemed important to determine whether livers from the species of animals used by man as food contained a tumor-inducing substance which, if present, might account for some of the human neoplasms of the alimentary tract, liver and biliary system. Accordingly, bovine and swine livers were each tested twice in 4 experiments. Swine myocardium also was tested. The solvent used for testing all extracts was tricaprylin; it was tested alone on 3 occasions, although the results are combined for brevity in reporting.

Each experiment was performed with organs from a number of cases, pooled in order to obtain enough extract to perform tests on satisfactory numbers of mice. Ten to 20 kgm. of tissue were generally used. (Table I).

TABLE I: TYPE OF TISSUE, WITH CHEMICAL DATA

Exper. No.	Tissue or organ extracted	Number of cases poolel	Weight of tissue extracted gm.	Weight of nonsaponifiable lipids gm.	Nonsaponifiable lipids, %
1	Human kidneys from cancer cases	61	10,555	31.1	0.29
2	Human kidneys from noncancer cases	58	10,019	49.2	0.49
3	Human spleens from cancer cases	54	10,481	63.8	0.61
4	Human spleen from noncancer cases	51	10,012	67.9	0.68
5	Human colons from cancer cases	54	10,016	24.2	0.24
6	Human colons from noncancer cases	51	10,484	23.4	0.22
7	Human hearts from cancer cases	63	14,105	21.4	0.16
8	Human hearts from noncancer cases	35	10,159	15.7	0.15
9	Human livers from cancer cases	18	20,000	103.0	0.51
10	Human livers from noncancer cases	15	20,000	94.7	0.47
11	Human livers from stillborn infants	40	4,108	19.1	0.46
12	Swine livers	—	10,000	52.0	0.52
13	Swine livers	—	10,000	49.9	0.50
14	Bovine livers	—	10,000	34.2	0.34
15	Bovine livers	—	9,959	53.7	0.54
16	Swine hearts	—	6,475	15.0	0.25
17	Tricaprylin controls	—	—	—	—

EXPERIMENTAL PROCEDURES

The methods of collecting the materials, chemical extraction, and biological testing, unless otherwise stated, resemble those used in previous experiments reported from this laboratory (18,22,25).

The fresh tissues were minced and preserved, either by freezing or by 1 or 2 volumes of ethanol, as soon as possible after necropsy. When the desired amount of material had been collected the extracts were made by the method previously described (18,22). Essentially it consisted of alkaline hydrolysis followed by extraction with ethylene dichloride, the saponification being repeated once. The extracts were prepared for injection by warming to 57° C. and stirring with tricaprylin to form a solution or suspension. Data on the extracts are presented in Table I.

Carcinogenic activity was tested by subcutaneous injection into mice of the C57 black strain. They were obtained from the Roscoe B. Jackson Memorial Laboratory or raised in our laboratory as the first generation offspring of mice obtained from there. This strain was used because of its low incidence of spontaneous tumors of all types, especially of subcutaneous sarcomas (12). In a few experiments, as indicated, extracts were tested both in C57 black mice and in the A strain of Little or in our own partly inbred albino strain, which was used in previous experiments reported from this laboratory in which materials of human and related origin were tested (19,22,23,25,28). Fifty mice or more, about equally divided as to sex, were used for each test if enough extract was available. A total of 1,044 mice was used in the 17 experiments. The mice were 5 to 12 weeks old at the time of injection. They were fed a mixed diet previously described (25).

The extracts were injected subcutaneously, 250 mgm. being given on 2 occasions 4 weeks apart for a total dose of 500 mgm. This was dissolved or suspended in 0.5 cc. of tricaprylin. The control mice were injected with 2.0 cc. of tricaprylin.

The experiments were terminated at the beginning of the 25th month or sooner. All animals were subjected to necropsy and all lesions suspicious of tumor were examined microscopically. A record was made of the approximate amount of extract that remained.

RESULTS

The three principal effects of the extracts were those of acute toxicity, causticity to tissues, and tumorigenic activity. The immediate toxicity was slight. No extract produced the severe, highly lethal reaction which others have exhibited (25,26). The causticity of the extracts was variable. In Experiment 12 and 14 the material was all given in one injection. Most of it sloughed out during the first month so that its carcinogenicity was not accurately determined. Consequently, new extracts were made, injected in Experiments 13 and 15 in divided doses, and found to be moderately caustic so that about half of it was extruded, but a partial test of potency was obtained. The extract used in Experiment 9 was likewise moderately caustic and approximately half of it was lost. The extracts injected in Experiments 3,4,5,6,10 and 11 were slightly caustic, resulting in about 25 per cent loss. The remaining extracts were comparatively noncaustic and good tests for carcinogenicity were possible. There was no relation of the degree of causticity of the extracts to their subsequent tumorigenicity.

In general the length of survival of the animals was good. Of the original 1,044 mice injected in 17 experiments, 925 lived more than 6 months when the first tumor occurred at the site of injection. Six months is used as the induction time in calculating the percentage yield.

Induced sarcomas.—Tumors were found at the site of injection and elsewhere in the mice as given

in Tables II, III and IV. Spindle and polymorphous cell sarcomas were found in the subcutaneous tissues in relation to remnants of the extracts. They infiltrated locally and grew rapidly. Some of them metastasized to the lungs. Transplantation experiments were not attempted but this procedure has previously been successful with similar tumors (22). These tumors resembled the sarcomas induced in mice by the carcinogenic hydrocarbons. They are known not to occur spontaneously in the strains of mice used, and they are considered to be induced tumors.

Sarcomas were induced by extracts of liver and spleen. The highest yield of tumors was obtained with material from noncancer livers. It induced 27 sarcomas in 46 C57 black mice (58.7 per cent) and 17 sarcomas in 45 mice of our albino strain (37.7 per cent), a combined incidence of 48.4 per cent. The figures for the corresponding cancer liver extract were lower (14.8 per cent for C57 blacks and 27.1 per cent for albinos; a total of 22.7 per cent). The difference in tumorigenic activity between these two extracts may well be explained by the much greater loss of material by sloughing through the skin in the mice injected with the cancer extracts. The first mouse with sarcoma died in the sixth month after injection, and most of the tumors occurred between the eighth and 13th months.

TABLE II: STRAIN, SEX, AND NUMBER OF MICE USED, THEIR SURVIVAL TIME, AND INDUCED SARCOMAS

Exper. No.	Strain	Mice used: Male	Female	Total	Survival of mice, months: 6	9	12	15	18	21	24	Induced sarcomas: No.	Yield %
1	C57 bl.	25	25	50	50	44	39	36	30	17	12	0	0
2	"	26	24	50	50	47	44	39	27	18	12	1	2.0
3	"	24	26	50	48	47	47	38	31	18	16	5	10.4
4	"	25	25	50	44	43	40	39	34	21	17	0	0
5	"	28	21	49	48	47	45	43	35	26	16	0	0
6	"	25	22	47	47	46	45	42	36	21	12	0	0
7	"	14	29	43	41	30	19	14	10	8	0	1	2.4
8	"	15	16	31	23	16	7	3	2	1	0	0	0
9A	"	25	25	50	27	24	12	6	6	5	0	4	14.8
9B	Albino	25	26	51	48	43	33	24	18	9	5	13	27.1
10A	C57 bl.	31	39	70	46	34	16	9	6	3	1	27	58.7
10B	Albino	25	25	50	45	38	23	14	11	3	2	17	37.7
11	C57 bl.	25	14	39	37	37	34	30	24	6	3	3	8.1
12	"	30	34	64	62	61	58	54	49	37	26	1	1.6
13	A	26	25	51	40	27	24	14	10	6	0	3	7.5
14	C57 bl.	27	37	64	63	63	56	52	47	38	27	0	0
15	A	27	24	51	32	21	13	10	7	0	0	0	0
16	C57 bl.	15	15	30	25	17	9	9	6	5	2	0	0
17	"	76	78	154	149	120	116	101	72	31	0	1	0.7
Totals		514	530	1044	925	805	680	577	461	273	151	76	

TABLE III: TYPE AND NUMBER OF TUMORS IN RELATION TO AGE AND SEX OF MICE IN WHICH THEY OCCURRED

Exper. No.	Type of tumor	Number of tumors, in months: 6	9	12	15	18	21	24	Tumors: In males	In females	Totals
1	Lymphatic			2		1			2	1	3
	Ovarian							1		1	1
2	Sarcoma					1			1		1
	Lymphatic				2	2			3	1	4
3	Sarcoma				1	1	2	1	1	4	5
	Lymphatic					1	1			2	2
4	Lung							1	1		1
	Lymphatic					1	2	2	2	3	5
	Ovarian							1		1	1
	Liver							1	1		1
5	Lung							2	1	1	2
	Lymphatic	1			1	2		3	4	3	7

Continued on next page

TABLE III (CONCLUDED): TYPE AND NUMBER OF TUMORS IN RELATION TO AGE AND SEX OF MICE IN WHICH THEY OCCURRED

Exper. No.	Type of tumor	Number of tumors, in months							Tumors		
		6	9	12	15	18	21	24	In males	In females	Totals
	Ovarian					1		2		3	3
	Liver				2			1	3	0	3
	Spleen						1			1	1
6	Lung						1	1	1	1	2
	Lymphatic		1			2	2	1	4	2	6
	Liver							1	1		1
7	Sarcoma	1							1		1
8											
9A	Sarcoma		1	3					4		4
	Lymphatic			1					1		1
9B	Sarcoma		2	7	1	1	1	1	7	6	13
	Lung				2		1	4	2	5	7
	Lymphatic	1				2	3	1	2	5	7
	Mammary gland				1		1			2	2
10A	Sarcoma		7	14	5	1			10	17	27
	Lymphatic		1							1	1
	Angioma						1		1		1
10B	Sarcoma	1	4	3	5	2	2		7	10	17
	Lung			1	2		1		1	3	4
	Lymphatic					1				1	1
	Mammary gland						1			1	1
11	Sarcoma					1	2		1	2	3
	Lymphatic			1	1	1	1		1	3	4
	Ovarian						1			1	1
12	Sarcoma			1					1		1
	Lung							2	2		2
	Lymphatic			1			2	2	1	4	5
	Liver							2	1	1	2
	Ovarian							1		1	1
13	Sarcoma			1	1			1	1	2	3
	Lung		2		1	2		1	3	3	6
	Lymphatic	1	1							2	2
	Liver				1				1		1
14	Lung			1			2	2	3	2	5
	Lymphatic						1			1	1
	Spleen							3	1	2	3
	Liver							1	1		1
	Lipoma							1		1	1
	Mammary gland			1						1	1
15	Lung		1	1			2		4		4
	Lymphatic			1					1		1
	Mammary gland	7	1							8	8
16	Lymphatic					1			1		1
17	Sarcoma		1							1	1
	Lung					1	1			2	2
	Lymphatic					2	5	3	5	5	10
		12	22	39	27	26	37	43	89	117	206

The sarcogenic factor was already present in the livers of stillborn infants as indicated by the occurrence of 3 sarcomas in 37 mice (8.1 per cent) despite loss of part of the material by extrusion.

Of great interest was the induction of tumors with an extract of hog liver in Experiment 13 where 3 sarcomas were found in 40 mice (7.5 per cent). In an earlier test of swine liver (Experiment 12) only 1 sarcoma occurred but this extract was not adequately tested because of great loss by sloughing. Extracts of cattle liver induced no tumors under similar conditions in Experiments 14 and 15.

The only other extract that induced a noteworthy number of tumors was that from cancer spleens with which, in Experiment 3, 5 subcutaneous sarcomas were induced in 48 mice, a yield of 10.4 per cent. The corresponding extracts from noncancer spleens gave negative results as did those from cancer kidneys, cancer and noncancer colons, noncancer hearts, and swine hearts. One sarcoma was found in each experiment with noncancer kidneys and cancer hearts. They are probably not significant in view of the fact that 1 sarcoma was found in 149 tricaprylin control mice (0.7 per cent).

A total of 76 sarcomas is recorded in Table II, of which 33 occurred in male mice and 43 in females, indicating no striking sex difference in sensitivity to the extracts. The induction time relationship of these tumors is given in Table III.

Other tumors.—In addition to the sarcomas at the site of injection already mentioned a few lymphosarcomas were also encountered, growing diffusely through the subcutaneous tissues in relation to the extract and with generalization. They are not included with the induced sarcomas but with the lymphatic tumors because lymphosarcomas sometimes grow in this manner in the absence of an injected subcutaneous carcinogen and it is not proved that they were induced. They were so few in number that for statistical purposes it does not matter much either way where they are tabulated. A few adenocarcinomas of the mammary gland were found in the A and the albino strains, but only once in the C57 black mice, in relation to the injected extracts. They occur spontaneously in the two former strains, they were few in number, their occurrence bore no relation to the degree of sarcogenicity of the extracts, and they are not counted as induced tumors.

Tumors were found at a number of sites distant from the point of injection. These included lymphatic, hepatic, pulmonic, splenic, ovarian, mammary and a few other types (Tables III and IV). They are not counted as induced because they did not exceed in type or number the reported incidence of spontaneous tumors in the C57 black strain (12), and the albino strain (22,25,28). Furthermore they were not more numerous in the experiments in which the extracts proved to be sarcogenic than in those in which no sarcomas were induced, or in the tricaprylin controls (Table V).

TABLE IV: SUMMARY OF INDUCED AND MISCELLANEOUS TUMORS

Type of tumor	Strain of mice: C57 black	A	Albino	Total No. of tumors
Sarcomas, at site of injection	43	3	30	76
Lung tumors: adenomas and carcinomas	14	10	11	35
Lymphatic and reticuloendothelial, including leukemias	50	3	8	61
Ovarian: adenomas, cystadenomas, and carcinomas	7	0	0	7
Liver: adenomas and hemangiomas	8	1	0	9
Spleen: hemangiomas	4	0	0	4
Mammary gland: carcinomas	1	8	3	12
Lipoma	1	0	0	1
Subcutaneous hemangioma	1	0	0	1

TABLE V: COMPARISON OF THE TUMORS AFTER THE INJECTION OF SARCOGENIC AND NONSARCOGENIC EXTRACTS

Strain of mice	Decree of sarcogenic acitivity of extracts	No. of experiments	No. of mice surviving at 6 mo.	Sarcomas: No.	Sarcomas: Yield, %	All other tumors: No.	All other tumors: Yield, %
C57 black	Percentage yield 0-2.4	10	453	3	0.7	64	14.1
	Percentage yield 7.4-58.7	4	158	39	24.7	10	6.3
	Tricaprylin controls	1	149	1	0.7	12	8.0
A	Percentage yield 0.0	1	32	0	0.0	13	40.6
	Percentage yield 7.5	1	40	3	7.5	12	30.0
Albino	Percentage yield 27.1-37.7	2	93	30	32.0	22	23.6
	Percentage yield 16.8*	2	101	17	16.8	31	30.7
	Percentage yield 0.0*	9	217	0	0.0	128	59.0
	Percentage yield 13.0**	12	154	20	13.0	41	26.6
	Percentage yield 0.0**	44	477	0	0.0	135	28.3

* Taken from (22), Experiments 1 and 3, and 5-9.

** Data from experiments reported in (25).

COMMENTS

The presence of a cancer-inducing factor has again been demonstrated in extracts of human livers from cancerous and noncancerous persons. The noncancer extract was the most potent, with respect to sarcogenicity, reported to date, while the activity of the corresponding cancer liver extract, although great, was not fully determined because of partial loss of the material by extrusion.

A review of the diagnoses in these persons yields no clew as to why the extracts were so active. In previous experiments (25) some evidence was obtained that carcinogenic activity in the extracts from liver was related to imbalances in the endocrine system. The extract used in Experiments 9 A and B was made from the pooled livers of 18 persons who had the following main diagnoses: Carcinoma of the stomach, 4 cases; carcinoma of the colon and lung, 3 cases each; carcinomas of the bladder and nasopharynx, 2 cases of each; 1 case each of carcinoma of the prostate, carcinoma of the bladder, hypernephroid carcinoma of the kidney, and lymphosarcoma. The 15 livers that were pooled to make the extract used in Experiments 10 A and B came from cases with the following main diagnoses: glomerulonephritis, nonspecific ulcerative colitis, diabetes mellitus, and coronary thrombosis, 2 cases of each; miliary tuberculosis, postpartum shock, hypertensive heart disease, arteriosclerosis, bronchopneumonia, cerebral hemorrhage, and postoperative pulmonary embolism, 1 case of each.

An extract of the livers of stillborn infants also induced tumors. This can be interpreted as evidence either for the endogenous nature of the active principle, or for transplacental transmission of an exogenous carcinogen from the mother. It can hardly be used as evidence that the activity is the result of the chemical procedures involved in preparation of the extracts because, as previously reported, 79 per cent of livers individually tested by identical methods were negative (25).

The observation that hog liver, but not that from cattle, showed evidence of activity despite incomplete testing because of loss of extract by extrusion may be of importance inasmuch as these species are used by man for food. Perhaps these results give scientific evidence for the wisdom of several races and religious groups in establishing ancient taboos. Obviously these experiments need repetition.

In this survey of organs and tissues other than liver, only cancer spleen showed evidence of considerable tumorigenic activity. In other experiments (27) 4 out of 62 extracts from lungs of adults and stillborn infants induced tumors. A survey of the organs of the body is now by no means complete, and perhaps some of the most promising sites were not tested. However, the field has been narrowed for the time being until more delicate and quicker methods of testing are available, and full efforts can be directed to liver and other organs. It appears that carcinogenic activity, as tested by the procedures reported here, is not a property of all protoplasm.

The results in Experiments 9 and 10, in which the same extracts were tested in the C57 black and our albino strains show that these two strains had approximately the same degree of sensitivity to the extracts. Sarcogenesis was not due to a strain-specific susceptibility. Neither was it the result of an extraordinarily high degree of strain susceptibility, because the C57 blacks have not shown such in the past.

SUMMARY

The presence of a tumor-inducing factor was again demonstrated in the nonsaponifiable lipid fraction of human liver from cancer-bearing and noncancerous persons. The combined percentage yields of sarcomas in mice of 2 strains was 22.7 and 48.4 respectively. A similar extract from the livers of stillborn infants was also sarcogenic strongly suggesting an endogenous nature for the active hepatic substance. Similar extracts of bovine livers were inactive but those from swine livers had sarcogenic activity. A survey of other human tissues including kidney, spleen, heart and colon from cancerous and noncancerous persons, and of swine hearts, revealed evidence of the tumorigenic factor only in extracts of spleen from persons who had cancer.

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Cancer Mortality among Males and Females in Denmark, England and Switzerland

I

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It is the object of this and subsequent articles to make such international comparisons of cancer incidence in Europe as may be possible on the basis of the available publications on cancer statistics, and on the material of Danish death certificates.

It is true that great caution must be used in employing death certificates for the purposes of cancer statistics, and we think that our own material is likely to strengthen this view. But as cancer registration in Europe at present is confined to a very few countries, cancer mortality is the only foundation available for international comparisons on a wider scale, and thus death certificates may still be used for directing attention to problems. The material of The Danish Cancer Registry will, however, be applied whenever possible.

Official publications from most countries are not very helpful in this respect. Whereas small, medically unimportant, administrative units, such as boroughs or small old cities, are dealt with separately in many statistical reviews, the reader of official statistics is far too often left in ignorance with regard to the number of cancers of each site. Even a moderate requirement of vital importance, as distribution of cancer cases of each site according to age groups of at least 5 years, is presented in only a few official publications. As it will be demonstrated, such distributions according to sex, site and age are of fundamental importance. Comparatively small expenditures on the publication of such figures, which presumably in many cases have been worked out already, might be very useful to research.

The value of death certificates varies with the quality of the medical services in the country concerned. No doubt some figures based on death certificates do not really bear out the conclusions built on them in the course of time. Even in countries medically advanced considerable numbers of death certificates from rural areas may be issued by laymen, and thus leave the accuracy of their mortality figures questionable.

In a former publication one of us (1) has discussed the reliability of Danish death certificates covering the period 1935-1939 here examined. Further facts on this question will be produced in due course.

An indication of the quality of death certificates from different countries may be obtained by working out the figures for the frequencies with which the diagnosis of "senile marasmus" is given as a cause of death. Though this view should not be carried too far, we think it advisable to quote these frequencies from Schinz, Rosin and Senti (11) together with the crude cancer mortalities given in official publications from various countries.

Table I gives the crude cancer mortality for various countries in the last years before the second World War, arranged in order of numerical magnitude. Although such figures must be supplemented with a study of the age distribution of the normal populations as well as of the cancer patients, they may still serve as starting points for investigations.

TABLE I

Country and Year	Crude cancer mortality per 100,000 living			Mortality from senile marasmus per 10,000 living, above the age of 60 1936-37, From Schinz *et al.* (11)
	Males	Females	Persons	
Switzerland, 1938	182	157	169	31
England-Wales, 1937	163	163	163	32
Denmark, 1938	130	164	147	63
Germany, 1938	138	155	147	90
Holland, 1938	137	149	143	52
Sweden, 1938	132	139	136	104
Norway, 1938	126	135	131	98
U. S. A., 1936	102	120	111	—
Finland, 1938	104	105	104	139
Italy, 1938	83	89	86	88
Rumania	—	—	—	447

The most outstanding feature of the figures in

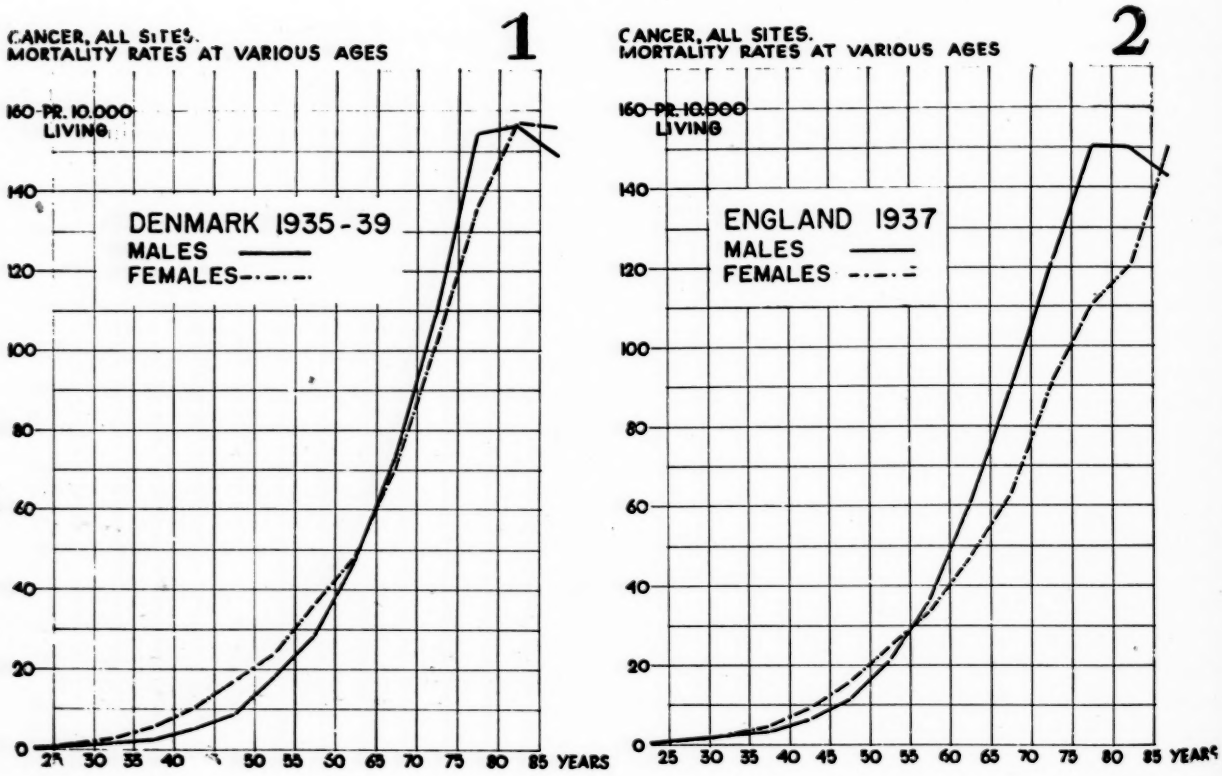
Table I is the difference in crude mortality rate between the sexes, in the first three countries on the list. Roughly speaking, mortality from cancer is highest among males in Switzerland. In England it is nearly equally heavy in the two sexes, whereas Denmark and the remaining countries show higher figures for crude cancer mortality among women than among men. However, the steep rise of cancer mortality with age make further analysis and calculations necessary as a basis for drawing any conclusions.

The mortality rates for cancer, all sites, for males and females, at various ages in Denmark, England, and Switzerland, for various years are shown in Figs. 1 to 5.

In comparing these diagrams the reader should keep in mind that the figures for Switzerland are given in 10 year groups, while the figures from the two other countries are subdivided in 5 year groups. It is also of importance to note that slight deviations of curves in the lower age classes will signify far heavier numerical differences, than in the older age classes where the populations are less numerous.

Consequently, Figs. 1, 2, and 3 do not directly show whether the sex ratios of cancer really correspond to the figures given in Table I, but they confirm the opinion that the general trend corresponds to the indications of the crude cancer mortality rates. Thus, the excess of cancers among females is pronounced in Denmark, while England shows a tendency to equilibrium between the sexes, and the male cancer mortality preponderance in Switzerland seems practically certain.

Figs. 4 and 5 indicate that these differences are due mainly to different male cancer mortalities in the countries concerned, because the corresponding curves show a regular and well pronounced difference in level, combined with similarity of shape. The curves for females can hardly be distinguished separately in the lower age classes, but as the following statistical analysis will show they contribute to the difference among the three countries by deviations opposite to those of the curves for males, though less pronounced. In most age groups the cancer mortality among females has been heavier in Denmark than in England and Wales, where, in its turn, it exceeds the corresponding figures for Switzerland.



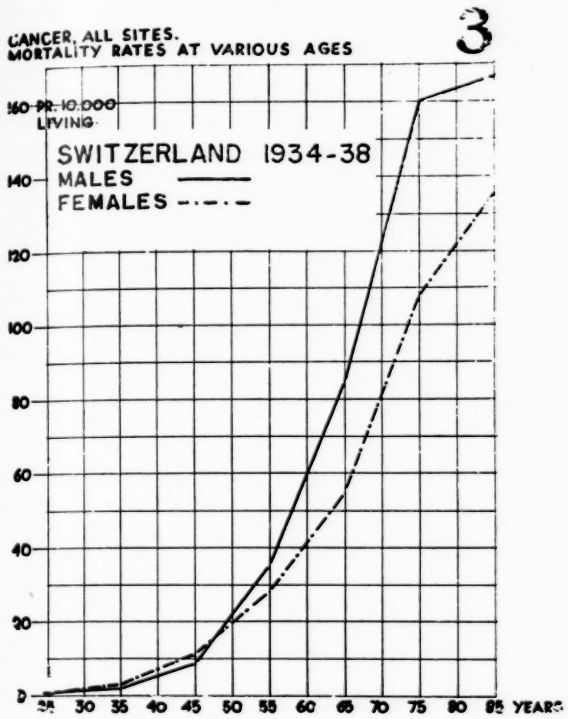
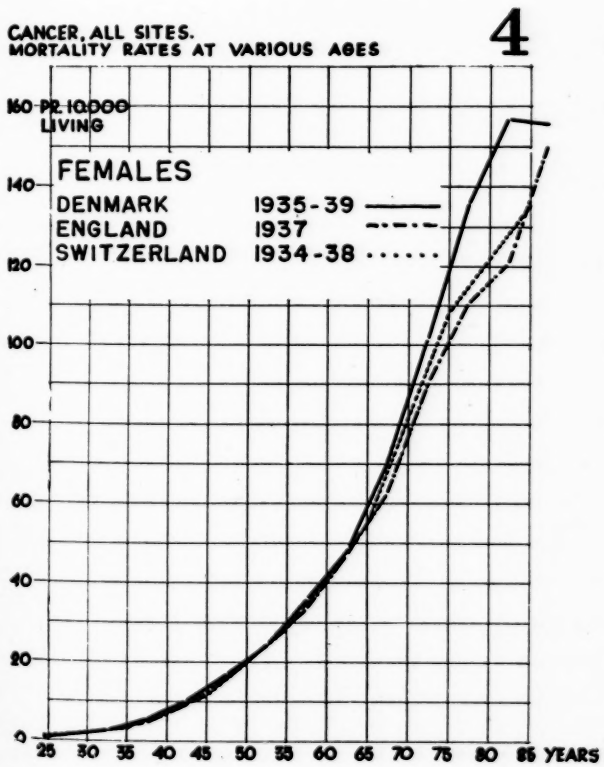


TABLE II: MORTALITY FROM CANCER OF ALL SITES PER 10,000 LIVING MALES

Age	Switzerland 1934-38	England and Wales 1937	Denmark 1935-39	England and Wales 1937	Denmark 1935-39
24	0.427	0.521	0.382	0.41	0.31
25-29				1.06	0.74
30-34	1.73	2.63	1.98	2.16	1.50
35-39				3.15	2.51
40-44	8.70	8.82	7.04	6.42	5.29
45-49				11.35	9.04
50-54	35.3	28.4	22.4	21.3	16.9
55-59				36.1	28.7
60-64	84.9	72.8	59.5	60.3	48.0
65-69				89.1	74.8
70-74	160.3	131.8	128.8	121.7	111.7
75-79				150.3	154.8
80-84	166.5	148.0	158.1	150.0	159.6
85-				142.6	154.3



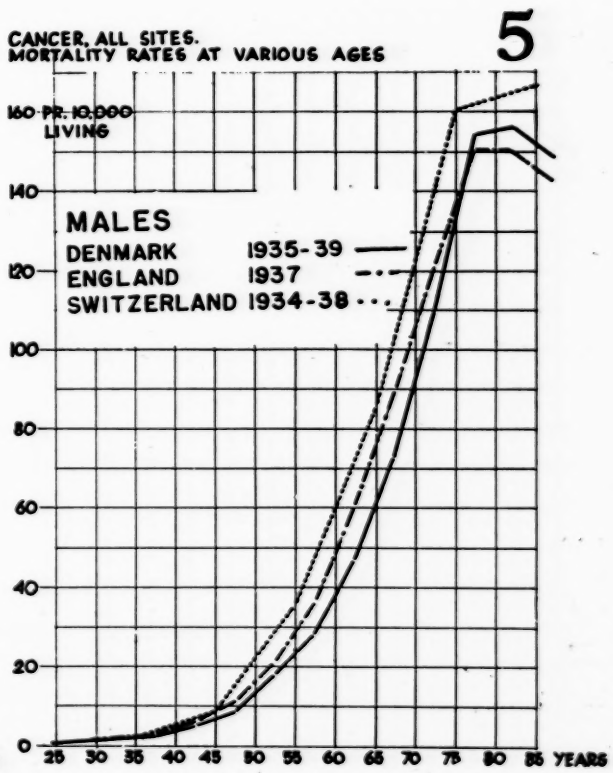


TABLE III: MORALITY FROM CANCER OF ALL SITES PER 10,000 LIVING FEMALES

Age	Switzerland 1934-38	England and Wales 1937	Denmark 1935-39	England and Wales 1937	Denmark 1935-39
24	0.395	0.426	0.449	0.31	0.28
25-29				0.93	1.23
30-34	2.68	3.16	4.27	2.16	2.76
35-39				4.83	5.89
40-44	10.1	12.5	13.8	9.22	10.39
45-49				15.9	17.6
50-54	27.8	29.0	30.3	25.0	24.4
55-59				33.2	37.2
60-64	54.4	53.8	59.0	47.0	49.5
65-69				62.6	71.1
70-74	107.9	98.5	116.6	91.0	103.3
75-79				110.5	136.6
80-84	135.5	134.1	159.7	120.8	159.6
85-				151.8	159.8

For testing the significance of these differences the values shown in the Tables II, III, IV, and V have been computed. The method applied is as follows:

Denoting the values for Switzerland, England-Wales, and Denmark respectively by the indices S, E, and D, and applying the notations: $n(x)$ for the total of the periods spent by individuals within the age groups $(x-2.5, x+2.5)$ or $(x-5, x+5)$ during the period considered.

$a(x)$ for number of cancer deaths in the age group $(x-2.5, x+2.5)$ or $(x-5, x+5)$ during the period considered.

$\mu(x)$ for estimated value of the cancer mortality rate for the age x. The comparison between e.g. Switzerland and England-Wales has been carried out by computing:

$$\tilde{\mu}(x) = \frac{a_S(x) + a_E(x)}{n_S(x) + n_E(x)}$$

$$u_I = \frac{\mu_S - \mu_E}{\sqrt{\tilde{\mu}(1-\tilde{\mu})\left(\frac{1}{n_S} + \frac{1}{n_E}\right)}}$$

$$u_{II} = 2\,\frac{\arcsin\sqrt{\mu_S} - \arcsin\sqrt{\mu_E}}{\sqrt{\frac{1}{n_S} + \frac{1}{n_E}}} \simeq 2\,\frac{\sqrt{\mu_S} - \sqrt{\mu_E}}{\sqrt{\frac{1}{n_S} + \frac{1}{n_E}}}$$

and, finally $u = \frac{1}{2}(u_I + u_{II})$

TABLE IV: TEST OF SIGNIFICANCE. MALES

	Values of u Sw. Engl.	Engl.-Denm.	Sw. Denm.	Significant difference? Sw.-Engl.	Engl-Denm.	Sw.-Denm.
24		2.69			yes	
25-29	—2.46	2.74	1.10	yes	"	
30-34		3.44			"	
35-39	—6.17	2.60	—1.55	"	"	
40-44		2.96			"	
45-49	—0.38	4.38	4.64		"	
50-54		5.84			"	yes
55-59	10.40	6.97	16.70	"	"	"
60-64		8.32			"	
65-69	9.62	6.85	17.15	"	"	"
70-74		3.32			"	
75-79	10.95	—1.04	10.12	"		
80-84		—1.52				"
85-	3.07	—1.20	1.28	"		

TABLE V: TEST OF SIGNIFICANCE. FEMALES

	Values of u Sw. Engl.	Engl.-Denm.	Sw. Denm.	Significant difference? Sw.-Engl.	Engl.-Denm.	Sw.-Denm.
24		0.88				
25-29	—0.87	—2.20	—1.30		yes	
30-34		—2.78			"	
35-39	—2.81	—3.18	—7.64	yes	"	yes
40-44		—2.52			"	
45-49	—6.77	—2.64	—8.58	"	"	"
50-54		0.74				
55-59	—2.00	—3.78	—3.47	"	"	"
60-64		—1.86				
65-69	0.62	—4.85	—3.70		"	"
70-74		—5.10			"	
75-79	5.00	—7.62	—3.61	"	"	"
80-84		—7.65			"	
85-	0.32	—1.00	—4.57		"	

Provided that the difference in cancer mortality between the countries here examined is due to chance alone, the variables u_1 and u_m will approximately be normally distributed with the mean value 0 and the standard deviation 1. According to an observation by A. Hald (6) this approximation can be improved by computation of the mean of u_1 and u_m, (in many cases u_1 and u_m are very nearly the same value and thus the method includes a check on the calculations). This variable u has therefore been used for the test of significance. Provided the correctness of the hypothesis that the difference in cancer mortality between the countries considered is due to chance alone, the probability that the numerical value of u exceeds 1.96, 2.58, and 3.29 is respectively 5 per cent, 1 per cent, and 0.1 per cent. Values of u numerically exceeding 1.96 are thus hardly in conformity with the hypothesis that the difference in cancer mortality is due to chance alone, and consequently such values are regarded as indicating a significant difference.

The results of the calculations of u are given in the Tables IV and V. A positive value of u indicates that the country first mentioned has shown the highest cancer mortality, whereas a negative value of u symbolizes the opposite proportion. It will be seen from the tables that in most cases the differences are significant. Where this is not the case it is mostly the question of young age groups with few cancer deaths or old age groups comprising few individuals.

SUMMARY

The object of this study was to make such international comparisons of cancer incidence in Europe as may be possible on the basis of the available publications on cancer statistics. In view of the lack of statistics of incidence it was necessary to use chiefly statistics of mortality. For the years around 1938, Switzerland, England and Denmark showed the highest crude cancer mortalities in Europe. In Switzerland, the crude cancer mortality was higher in males. In Denmark, as in most other countries, the highest cancer mortality was among females. England was in an intermediate position. These differences were found from analysis of the statistics to be due mainly to deviations in male cancer mortality. The international variations in female cancer mortality, although statistically significant, are smaller and directly opposite to the variations in male cancer mortality, thus tending to increase the international differences mentioned.

ACKNOWLEDGMENT

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Cancer Mortality among Males and Females in Denmark, England and Switzerland

II. Danish Towns and Rural Areas

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In the preceding paper (1) we have reported that in the years about 1938, Switzerland showed the highest crude cancer mortality rate in Europe, with the highest rate in males. England, on the other hand, with the second highest crude cancer mortality rate, tended to show equilibrium between the sexes. Denmark, the third country studied, showed a higher cancer mortality rate among females. These differences were due mainly to variations in the male cancer mortality, although smaller differences in female cancer mortality tended to increase the differences demonstrated between the cancer mortality rates of the sexes.

In this paper it is our intention to demonstrate similar differences in cancer mortality in Danish towns and rural areas. Further analysis of both groups of statistics might serve to elucidate the causal factors.

A fundamental difficulty in cancer statistics is the lack of uniformity in the material collected; some diagnoses have been made by hospital examinations, others by general practitioners. Table I, covering the years 1931 to 1944 (all the years from which material has been used for the present investigations) gives the percentage of deaths from cancer in hospitals in the capital (Copenhagen), Danish provincial and rural areas, and in the hospitals throughout Denmark. It is true that a number of fatal cases for which death certificates are issued by general practitioners have been examined previously in hospitals. However, this number varies with particular diagnoses and also varies through the years under review in this study. An exact estimate would require a preceding period of some length during which The Danish Cancer Registry had been working under constant conditions. The cancer mortality rates for males and females at various ages in these areas are shown in Figs. 1 to 5.

Fig. 3 showing cancer mortality rates in Danish rural areas during the years 1935 to 1939 appears to correspond closely to Fig. 1 of the preceding paper which presents the total cancer mortality

TABLE I: CANCER DEATHS IN DANISH HOSPITALS (INCLUDING SARCOMAS. OTHER TUMORS EXCLUDED)

Year	Copenhagen		Provincial towns		Rural areas		Denmark	
	Total no.	No. in hosp. %	Total no.	No. in hosp %	Total no.	No. in hosp. %	Total no.	No. in hosp. %
1931	1,155	64.4	1,153	38.5	2 715	29.5	5,024	39.6
1932	1,264	64.2	1,189	38.4	2,786	30.7	5,239	40.5
1933	1,209	62.3	1,286	42.2	2,842	31.9	5.337	41.3
1934	1.235	64.0	1,296	42.5	2,752	31.0	5.283	41.5
1935	1,287	65.1	1,325	40.4	2,789	34.9	5.401	43.5
1936	1,330	66.9	1,336	45.7	2,946	33.2	5,612	44.2
1937	1,381	66.5	1,334	47.3	2.782	34.5	5.497	45.6
1938	1,356	67.6	1,349	47.0	2,854	35.6	5,559	46.2
1939	1,326	70.4	1,348	52.2	2,785	36.7	5,459	48.7
1940	1,411	70.9	1,338	57.1	2,919	42.3	5,668	52.9
1941	1,373	71.7	1,510	57.9	2.869	42.8	5,752	53.7
1942	1.476	68.2	1,508	55.6	2,897	40.2	5,881	51.1
1943	1,513	67.9	1,610	51.6	3,017	40.1	6,140	50.0
1944	1,542	54.0	1,633	43.8	3,088	36.6	6,263	42.8

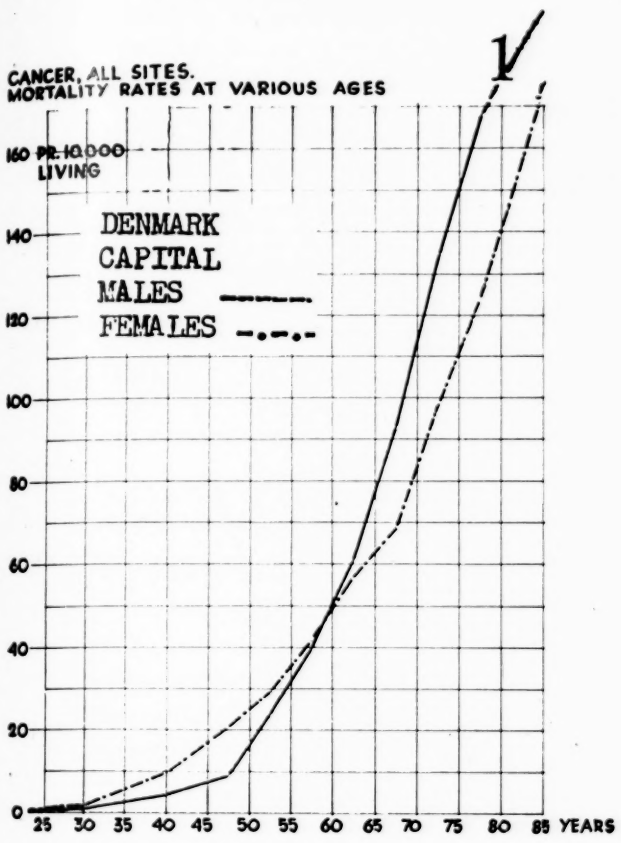


TABLE II: DENMARK, 1935-39. MORTALITY FROM CANCER PER 10,000 LIVING. ALL SITES

Age	Copenhagen	Provincial towns	Rural areas	Copenhagen	Provincial towns	Rural areas
		Males			Females	
Up to 24	0.299	0.361	0.294	0.266	0.221	0.316
25 " 29	0.746	0.373	0.902	1.09	1.41	1.22
30 " 34	1.42	1.53	1.53	2.76	3.09	1.58
35 " 39	3.12	2.64	2.16	6.38	6.13	5.48
40 " 44	6.71	4.38	5.03	12.8	10.5	8.98
45 " 49	9.43	9.76	8.51	20.7	17.8	15.7
50 " 54	23.8	15.7	14.4	28.2	23.2	22.9
55 " 59	39.0	25.6	25.7	41.9	36.1	35.0
60 " 64	58.5	50.2	42.9	53.4	52.2	45.9
65 " 69	89.0	79.8	67.5	67.4	74.9	71.0
70 " 74	128.6	113.4	105.3	97.9	107.2	104.2
75 " 79	173.4	147.7	152.1	128.4	133.3	142.8
80 " 84	183.8	162.2	153.0	168.7	162.7	153.8
85 and over	176.1	146.8	152.4	161.9	170.1	154.2

rates for Denmark. Fig. 2, showing the cancer mortality rates for provincial towns, does not deviate much from this pattern. However, Fig. 1, presenting the cancer mortality rates for the capital (Copenhagen), displays some similarity to the graphs of rates for England and Switzerland (see Figs. 2 and 3 of the preceding paper) showing considerable excess of males in the higher age groups. Figs. 4 and 5, for comparison, present the cancer mortality rates of males and females separately for the three areas under consideration (capital, provincial towns and country districts). It appears from these figures that the differences between the rates for the capital as compared with the rates for Danish rural areas during the years 1935 to 1939 are ascribable mainly to the cancer mortality rates for males.

The graphs for the Danish female cancer mortality rates, contrary to what was seen in the international comparison, display among themselves the same trends as the graphs for the males in the three areas under examination, and thus to a slight degree tend to diminish the differences between the rates for the capital and rural areas with regard to the proportion of cancer mortality of the two sexes.

Computations were carried out according to the method described in our previous paper (1). The results of these computations, given in Tables II and III, confirm these conclusions.

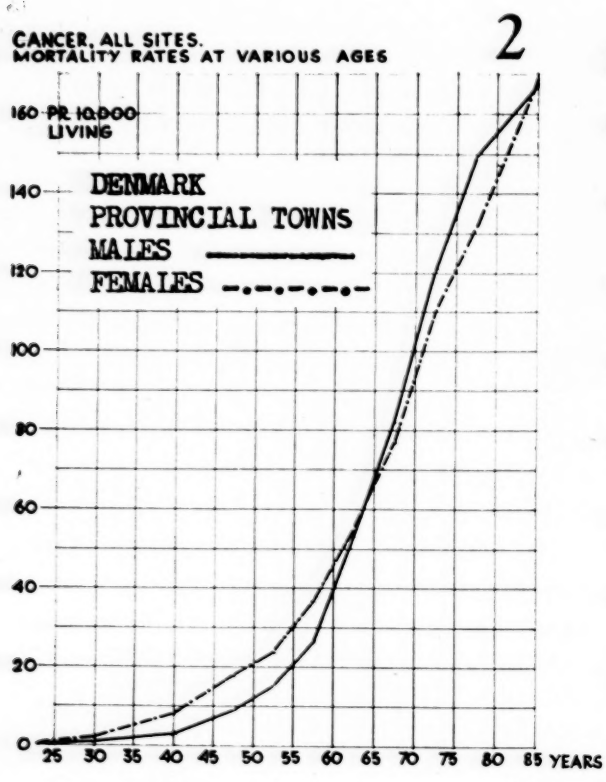
TABLE III: DENMARK, 1935-39. CANCER MORTALITY ACCORDING TO AREA

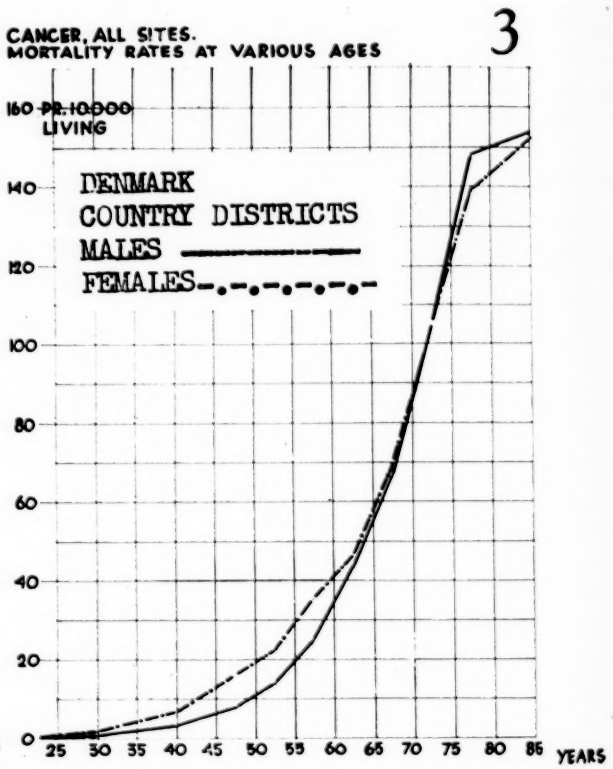
TEST OF SIGNIFICANCE. MALES

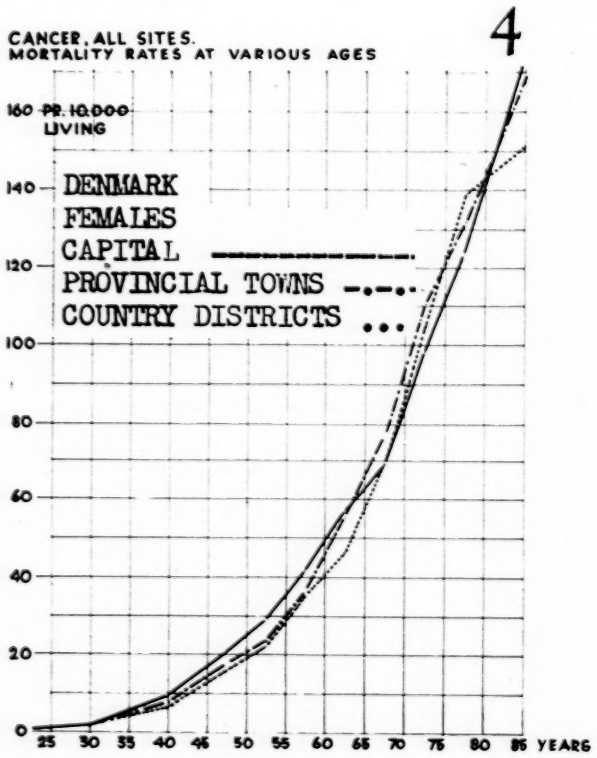
Age	Values of u: Copenhagen—prov. towns	Prov. towns—rural areas	Copenhagen—rural areas	Significant difference? Copenhagen—prov. towns	Prov. towns—rural areas	Copenhagen—rural areas
Up to 24	—0.68	0.94	—0.06			
25 " 29	1.63	—2.32	—0.67		yes	
30 " 34	—0.28	0.01	0.32			
35 " 39	0.80	1.03	2.00			yes
40 " 44	2.66	—0.92	2.22	yes		"
45 " 49	—0.27	1.22	0.92			
50 " 54	4.38	0.92	6.28	"		"
55 " 59	5.20	—0.06	6.20	"		"
60 " 64	2.21	2.60	5.28	"	"	"
65 " 69	1.72	3.05	4.95		"	"
70 " 74	1.96	1.38	3.60	"		"
75 " 79	2.26	—0.52	2.17	"		"
80 " 84	1.22	0.74	2.05			"
85 and over	1.12	—0.32	1.03			

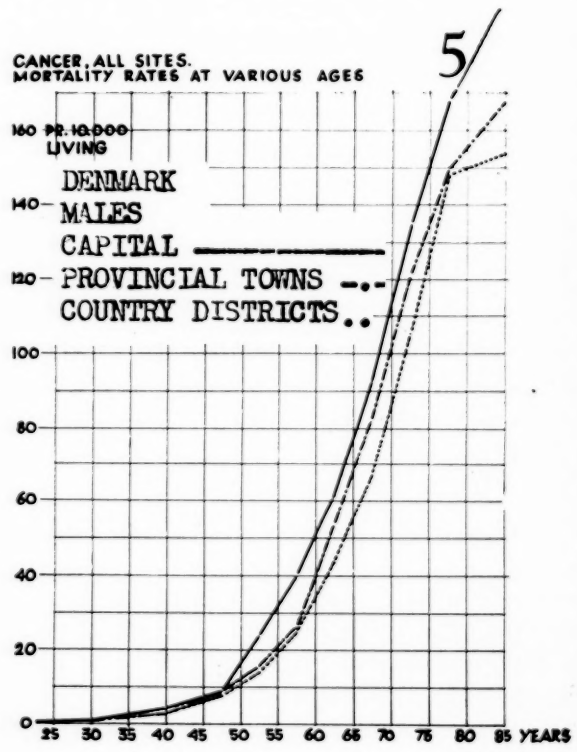
TEST OF SIGNIFICANCE. FEMALES

	Values of u: Copenhagen—prov.	Prov. towns—rural	Copenhagen—rural	Significant difference? Copenhagen—prov.	Prov. towns—rural	Copenhagen—rural
Up to 24	0.60	—1.48	—0.70			
25 " 29	—0.95	0.63	—0.42			
30 " 34	—0.62	1.10	0.42			
35 " 39	0.30	0.92	1.30			
40 " 44	1.99	1.54	3.90	yes		yes
45 " 49	1.78	1.54	3.72			
50 " 54	2.54	0.20	3.18	"		"
55 " 59	2.22	0.50	3.14	"		"
60 " 64	0.38	2.22	2.74		yes	"
65 " 69	—1.73	1.00	—0.96			
70 " 74	—1.53	0.55	—1.20			
75 " 79	—0.60	—1.25	—1.94			
80 " 84	0.48	0.80	1.40			
85 and over	—0.41	0.97	0.46			


2
CANCER, ALL SITES.
MORTALITY RATES AT VARIOUS AGES
160 PR. 10.000 LIVING
DENMARK
PROVINCIAL TOWNS
MALES
FEMALES
0 20 40 60 80 100 120 140
25 30 35 40 45 50 55 60 65 70 75 80 85 YEARS


3
CANCER, ALL SITES.
MORTALITY RATES AT VARIOUS AGES
160 PR. 10.000 LIVING
DENMARK
COUNTRY DISTRICTS
MALES
FEMALES
0 20 40 60 80 100 120 140
25 30 35 40 45 50 55 60 65 70 75 80 85 YEARS


4
CANCER, ALL SITES.
MORTALITY RATES AT VARIOUS AGES
160 PR. 10.000 LIVING
DENMARK
FEMALES
CAPITAL
PROVINCIAL TOWNS
COUNTRY DISTRICTS
0 20 40 60 80 100 120 140
25 30 35 40 45 50 55 60 65 70 75 80 85 YEARS


5
CANCER, ALL SITES.
MORTALITY RATES AT VARIOUS AGES
160 PR. 10.000 LIVING
DENMARK
MALES
CAPITAL
PROVINCIAL TOWNS
COUNTRY DISTRICTS
0 20 40 60 80 100 120 140
25 30 35 40 45 50 55 60 65 70 75 80 85 YEARS

SUMMARY AND CONCUSIONS

Comparisons of cancer mortality of males and females in Danish towns and rural areas show a tendency to excess of mortality among males in the capital. This excess is not found in the rural districts. The differences between the areas examined show some similarity to those previously demonstrated for Switzerland, England and Denmark. With respect to these differences Switzerland and England correspond to the Danish capital (Copenhagen), and Denmark as a whole to its own country districts. In both cases the differences are mainly ascribable to differences in male mortality from cancer. On the other hand, whereas deviations of the cancer mortality rates for females tend to accentuate the international differences between rates for the two sexes, the rates for females within Denmark show a slight tendency to correspond to the rates for males.

REFERENCE

1: Clemmesen, J., and Busk, Th. Cancer Mortality among Males and Females in Denmark, England, and Switzerland. I. Cancer Research, 7:281-285.

Immunological Studies of Hydrocarbon-Protein Conjugates*

I. Precipitin Reactions**

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The present immunological studies are extensions of preliminary investigations (4) in which it was demonstrated that anthracene and 1,2,5,6-dibenzanthracene when chemically combined with proteins formed conjugates which exhibited haptenic activity. The principles and objectives of the research have been discussed in earlier publications (4, 9); in summary, the main purpose is to determine whether or not the injection of these conjugates into experimental animals will produce antisera capable of preventing the action of carcinogenic hydrocarbons. An indication of such a possibility was obtained in earlier work (9) and made worth-while the synthesis of a series of isocyanates (3, 8) of several polynuclear aromatic hydrocarbons and the formation of conjugates by the reaction of these compounds with various proteins (5-7, 10). In order to guide further studies of the protective properties of the conjugates, it was considered advisable to conduct a thorough investigation of their serological properties.

The hydrocarbon-protein conjugates with which the present studies are concerned were prepared under such experimental conditions that the protein component would undergo the least possible alteration. Thus, the serological results should reflect changes due only to the hydrocarbon group attached to the protein molecule. This condition is particularly important with this type of conjugate because hydrocarbon haptenes are relatively weak (11, 13) and exert most of their influence as a result of steric factors. The serological influence of the carbamido linkage,—NHCONH—, joining the hydrocarbon to the protein is unknown.

Serum albumins were found to be the most suitable proteins for use in the preparation of the conjugates because they would withstand the experimental conditions required for the introduction of the requisite number of hydrocarbon groups by the reaction of the isocyanates with the E-amino groups of the protein molecule. Furthermore excellent methods of spectrophotometric analysis for the determination of the amount of hydrocarbon in the conjugates made possible the isolation of highly purified preparations. Evidence obtained by such means (6) indicated that the methods used for the fractionation of the conjugates had removed any serum albumin which had not reacted with the isocyanate. The pronounced fluorescence of such conjugates in ultraviolet light also led to extensions of the research to problems in which this property could be utilized for "tracer" studies (1, 2).

Serological results were obtained by customary visual evaluation of the precipitates obtained by mixing solutions of antigens and antisera. The first paper of this series is a summary of the main results of such tests. More exact information was obtained by micro-Kjeldahl analyses of washed precipitates produced by these interactions (paper II of this series).

*These investigations were supported in part by grants from The International Cancer Research Foundation. Correspondence concerning this and the following papers should be set to H. J. Creech, Lankenau Hospital Research Institute, Philadelphia 30, Pa.

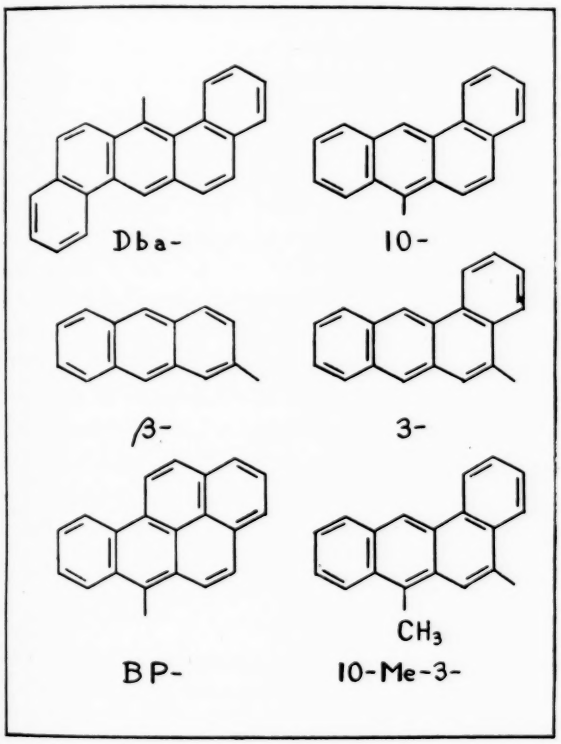
** Some of the results reported in this paper were presented at the 35th annual meeting of the American Association for Cancer Research, Inc., at Boston, Mass., 1942.

*** Certain phases of the work presented in this and the following papers were conducted by E. L. Oginsky in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the University of Maryland, College Park, Md., under the direction of H. J. Creech and O. N. Allen. Present address of E. L. Oginsky: Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

EXPERIMENTAL

The conjugates were prepared,[1] purified and analyzed for hydrocarbon content by the usual methods (5-7). Dependent on the experimental conditions, varying numbers of hydrocarbon groups could be introduced into the proteins. The proteins and conjugates together with the number of hydrocarbon groups per molecule of protein and a descriptive code are listed in Table I. As illustration of the meaning of the code, 10-HSA-14 stands for 1,2-benzanthryl-10-carbamido horse serum albumin containing *14* benzanthryl groups per molecule of protein.

The structural formulas of the prosthetic groups may be represented as shown in Fig. 1.



The carbamido linkage, —NHCONH—, is formed by the reaction of the aromatic isocyanate, ArNCO, with the ϵ-amino group of the lysine side chains of the proteins (7). A complete reaction involving all the ϵ-amino groups of any one protein was not found to occur under the experimental conditions employed (5-7); more extensive conjugations than those listed above usually could not be accomplished without denaturing the protein component.

Table I: List of Proteins, Hydrocarbon-Protein Conjugates and Code Designations

Protein or Conjugate	Code (hycb.-prot.-no. of hycb. groups)
Horse serum albumin	HSA
Bovine serum albumin	BovA
Human serum albumin	USA
Horse serum γ-pseudoglobulin	HSG
Egg albumin	EA
1,2-benzanthryl-10-carbamido horse serum albumin	10-HSA-12
1,2-benzanthryl-10-carbamido horse serum albumin	10-HSA-14
1,2-benzanthryl-10-carbamido horse serum albumin	10-HSA-16
1,2-benzanthryl-10-carbamido horse serum albumin	10 HSA-26
1,2-benzanthryl-10-carbamido horse serum albumin	10-HSA-38
1,2-benzanthryl-3-carbamido horse serum albumin	3-HSA-8
1,2-benzanthryl-10-carbamido bovine serum albumin	10-BovA-15
1,2-benzanthryl-10-carbamido bovine serum albumin	10-BovA-31
1,2-benzanthryl-10-carbamido human serum albumin	10-USA-29
1,2-benzanthryl-10-carbamido horse serum γ-ps-globulin	10-HSG-9
1,2-benzanthryl-10-carbamido horse serum γ-ps-globulin	10-HSG-15
1,2-benzanthryl-3-carbamido horse serum γ-ps-globulin	3-HSG-5
1,2-benzanthryl-3-carbamido horse serum γ-ps-globulin	3-HSG-13
1,2-benzanthryl-10-carbamido egg albumin	10-EA-1
1,2-benzanthryl-3-carbamido egg albumin	3-EA-1
3,4-benzpyrenyl-5-carbamido horse serum albumin	BP HSA-4
3,4-benzpyrenyl-5-carbamido horse serum albumin	BP HSA-19
3,4-benzpyrenyl-5-carbamido bovine serum albumin	BPBovA-4
3,4-benzpyrenyl-5-carbamido bovine serum albumin	BPBovA-19
1,2,5,6-dibenzanthryl-9-carbamido horse serum albumin	DbaHSA-19
1,2,5,6-dibenzanthryl-9-carbamido bovine serum albumin	DbaBovA-21
10-methyl-1,2-benzanthryl-carbamido horse serum albumin	10-Me-3-HSA-16
β-anthryl-carbamido horse serum albumin	β-HSA-9

These experiments were conducted during the period 1940-46 and were subjected to several interruptions due to war-time conditions. Inasmuch as a considerable portion of the serological investi-

[1] The required quantities of 1,2-benzanthracene, 1,2,5,6-dibenzanthracene, the isocyanates therefrom and a preparation of 1,2-benzanthryl-10-carbamido horse serum albumin were made by Edward Walton and Dorothy Campbell Svirbely. Several spectrophotometric determinations were conducted by Dr. R. N. Jones and by Daniel Draper.

gations was repeated several times, and then correlated with later studies, it is expedient to summarize the findings of the preliminary work (Series I and II). Details of the procedures of immunization and of serological testing were essentially similar to those described later (Series III). In the first 2 series, a total of 450 mgm. of protein or conjugate was administered to each rabbit by intravenous and intraperitoneal routes. At least 2 rabbits were injected with each antigen.

Series I and II.— In the following tables and discussions are recorded the pertinent observations and conclusions reached in these preliminary studies. The antigens used for immunization in the first series were HSA, 10-HSA-12, 10-HSG-15, 3-HSA-8 and 3-HSG-13.

Table II: Test Antigens and Precipitin Reactions, Series *I*

Antisera	HSA	HSG	10-HSA 12	10-HSG-9	3-HSA-8	3-HSG-5	10-EA-1	3-EA-1
HSA	S		S		S		—	
HSG	—	S	—	S		S		—
10-HSA-12	S		S	—	S			
10-HSG-15	—	S	W	S	—		—	
3-HSA-8	S	—	S		S	—		—
3-HSG-13			—		W	S		—

S = Satisfactory precipitin test.

W = Weak precipitin test.

— = No reaction.

The test antigens were HSA, HSG, 10-HSA-12, 10 HSG 9, 3 HSA 8, 3-HSG-5, 10-EA-1 and 3-EA-1. In Table II, S stands for a satisfactory precipitin test, W for a weak test, and — for no reaction. Serological tests involving all antigens and antisera were not conducted.

It is evident from a study of Table II that the introduction of 1,2-benzanthracene into the proteins did not destroy the immunological specificity of the protein. The antisera from every immunizing antigen reacted with every test antigen containing the same protein whether or not that protein had attached hydrocarbon, e.g. antisera toward HSA, 10-HSA-12 and 3-HSA-8. It is highly improbable that any reaction such as that of the antisera towards HSA with the 10-HSA-12 test antigen occurred because of the presence of unaltered HSA in the 10-HSA-12 preparation. Fractionation experiments (6) indicated the purity of the conjugates.

The most important of the reactions with which these studies are concerned are those involving only the prosthetic group activity of the conjugates. For these reactions, it is necessary to examine the results of tests in which the protein components of the conjugates used for immunization and for test antigens were different. In the first series, there were two such positive reactions. The antiserum toward 10-HSG-15 reacted weakly with 10-HSA-12 whereas neither the HSA nor the 3-HSA-8 antigen gave any test with that antiserum. This indicates that the 1,2-benzanthryl-10 radical has altered the antibody-producing properties of horse serum globulin; thus, the injection of 10-HSG-15 has led to the formation of antibodies capable of reacting with a different protein (HSA) with attached 1,2-benzanthryl-10-radicals but not with a horse serum albumin conjugate containing 1,2-benzanthryl-3-radicals. Two interpretations may be made for the lack of this reaction: (a) that is a sharp serological differentiation between conjugates containing 1,2-benzanthracene attached at the 10- or 3- position or (b) that the number of 1,2-benzanthryl-3-radicals contained in the test conjugate is below the critical level for prosthetic group activity. In view of the lack of reaction between test antigen 10-HS-9 and the antiserum toward 10-HSA-12, and in view of evidence to be discussed later, the second interpretation is more probable. Test antigens, 10-EA-1 and 3-EA-1, showed no prosthetic group activity presumably because of the low content of 1,2-benzanthracene in the egg albumin molecule.

To expand these observations, the experiments of Series II were conducted. The antigens used for immunizing the rabbits were HSA, 10-HSA-14, BP=HSA-4, 10-BovA-15 and BPBovA-4. The test antigens are listed in Table III. The results are recorded as the average maximum degree of precipitation observed in a large number of serological tests using 0.1 cc. of antiserum and 0.2 cc. of test antigen within the range of 40 to 200 γ of protein per 0.2 cc. of test antigen. The degree of precipitation is recorded as follows: —, no reaction; tr, a turbid appearance with only a slight precipitate, ±, a small amount of precipitate; +, a greater amount of precipitate and so on up to the maximum ++++ of a heavy precipitate.

It may be noted that the bovine serum albumin conjugates did not stimulate the production of good antisera; in certain cases, they acted as satisfactory test antigens although in this series some non-specific

TABLE III: TEST ANTIGENS AND PRECIPITIN REACTIONS, SERIES II

Antisera	HSA	10-HSA-14	BPHSA-19	10-BovA-15	10-BovA-31	BPBovA-4	BPBovA-19	BovA
Control	—	—	—	—	—	—	—	—
HSA	++++	+++	++±	±	±	tr	±	tr
10-HSA-14	+++	+++	++±	+	++	tr	+	tr
BPHSA-4	++±	+++	++	±	+±	tr	+	tr
10-BovA-15	tr	+	tr		++		+	±
BPBovA-4	tr	±	tr		+		+	±

— = No reaction.
tr = Turbidity, with slight precipitate.
± = Small amount of precipitate.
+ to ++++ = Definite to large amount of precipitate.

precipitation occurred between bovine serum albumin test antigens and antisera toward horse serum albumin and toward conjugates prepared from that protein.[2] Again, the serological specificity of the protein component was maintained after conjugation with the hydrocarbons. Apparently for purposes of immunization, 4 benzpyrenyl groups per molecule of albumin barely were sufficient to produce antisera showing activity directed towards the prosthetic group. On the other hand, a greater number of groups was required in the test antigen, as BP-BovA-4 was negative in action (when compared with control HSA antiserum), whereas BPBovA-19 was positive, with antisera toward BP-conjugates prepared from horse serum albumin. Stronger reactions were noted when the test antigen had an even higher hydrocarbon content, e. g. 10-BovA-31 gave the best prosthetic group reactions.[3] Hydrocarbon specificity was not particularly sharp as is indicated by the positive reactions of test antigen 10-BovA-31 with antisera toward 10-HSA-14 and BPHSA-4. Other examples in the table support this contention.

Series III.—Albino rabbits, weighing approximately 3 pounds each, were injected with a total of 450 mgm. of antigen as follows: HSA (2 rabbits), BP HSA-4 (2 rabbits), 10-HSA-38 (3 rabbits), Dba HSA-21 (3 rabbits). Three weeks of intravenous injections 3 times a week were followed by 2 weeks of intraperitoneal injections also 3 times a week. Each rabbit received 30, 60, 90 120, 180 mgm. of antigen in the successive weeks of treatment. The solutions contained 10 mgm. of antigen per cc. One rabbit from each group was exsanguinated by sterile heart puncture 10 to 13 days after the last injection. The remainder of the rabbits received 3 additional intraperitoneal injections (totalling 90 mgm.) commencing 1 week after the final injection of the preceding series; these rabbits were exsanguinated in the same manner as the first series. The antisera were obtained and stored in the refrigerator in sterile bottles with a few drops of merthiolate (Lilly) as preservative.

For the precipitin tests, the antisera were diluted with an equal volume of 0.85 per cent sodium chloride solution; 0.1 cc. of the diluted antiserum was used in the serological tests. The solutions of test antigen were made by diluting the stock solutions (10 mgm. per cc.) with 0.85 per cent sodium chloride solution; 0.2 cc. of each dilution of antigen was added to 0.1 cc. of diluted antiserum. The tubes were incubated at 37° C. for an hour and then placed in the refrigerator overnight. Readings were made the following morning.

The results of the serological tests are listed in Tables IV to VI. No significant differences in antibody content were noted between the groups that had received 450 mgm. of antigen and those that had received 540 mgm. of antigen. All values are averages of several tests employing the antisera from all the rabbits in each group. In Table IV are the results of the intreactions of antisera with the homologous antigen, *i.e.* the antigen used for the production of the antiserum. The dilution of antigen represents a dilution of stock antigen containing 10 mgm. per cc. The actual amount of antigen used in the test is given in the next column. The most active antisera were those prepared from unaltered horse serum albumin. Of the conjugates, 10-HSA-38 gave the strongest serological reactions.

Tables V and VI list again the average maximum degree of precipitation as +, ++, etc.; the number of micrograms of antigen used per 0.05 cc. of undiluted antiserum at this zone is given in brackets. It is evident from Table V that antisera toward 10-HSA-38 displayed considerable activity. The presence of 38 hydrocarbon groups per molecule

[2] Bovine serum albumin and conjugates therefrom had been prepared by ammonium sulfate fractionation. In later work, the bovine serum albumin employed had been obtained by alcohol fractionation. This latter product was supplied by Dr. E. J. Cohn.

[3] The theoretical limit of hydrocarbon content in the serum albumins of the horse and cow is 66 groups; in practice, the highest level obtained without denaturing the protein was 38 groups.

TABLE IV: DEGREE OF PRECIPITATION OF ANTISERA WITH HOMOLOGOUS ANTIGENS, SERIES III

Dilution of antigen	γ of Antigen per 0.05 cc. of antiserum	Antigen	HSA	BPHSA-19	10-HSA-38	Dba-HSA-21
		Antiserum	HSA	BPHSA-4	10-HSA-38	Dba-HSA-21
1:10	200		++	++	±	f. tr.
1:50	40		++	+	+±	tr.
1:100	20		+++	+	++	±
1:200	10		+++±	++	++±	tr.
1:400	5		++++	+	++±	tr.
1:1000	2		++±	tr.	++	—
1:2000	1		+	—	±	—
1:3000	0.6		±	—	tr.	—

TABLE V: AVERAGE MAXIMUM DEGREE OF PRECIPITATION OF ANTISERA WITH TEST ANTIGENS CONTAINING HOMOLOGOUS PROTEIN AND HETEROLOGOUS HYDROCARBON

	Antiserum (0.05 cc.)			
Antigen	HSA	BPHSA-4	10-HSA-38	Dba HSA-21
HSA		+± (200)	++± (5)	+± (2)
BPHSA-19	++± (200)*		++± (10)	+ (1)
10-HSA-38	tr. (80)	+ (200)		tr. (3)
Dba HSA-21	± (5)	tr. (150)	+± (10)	

* The figures in parentheses are the number of micrograms of antigen.

TABLE VI: AVERAGE MAXIMUM DEGREE OF PRECIPITATION OF ANTISERA WITH TEST ANTIGENS CONTAINING HETEROLOGOUS PROTEIN

	Antiserum (0.05 cc.)			
Antigen	HSA	BPHSA 4	10-HSA-38	DbaHSA-21
BPBovA-19	—	± (2000)*	±+ (1000)	± (500)
10-BovA-15	—	tr. (250)	++ (50)	tr. (200)
DbaBovA-21	—	+(2000)	+ (2000)	± (2000)

* The figures in parentheses are the number of micrograms of antigen.

of protein caused a considerable modification of the serological characteristics of the serum albumin portion of the molecule when employed as a test antigen (see Table III for 10-HSA-14). This conjugate was more satisfactory for the production of antisera showing haptenic action than the other two conjugates which contained fewer hydrocarbon groups. The haptenic activity of the conjugates is demonstrated by the results in Table VI obtained by testing the antisera with conjugates prepared from a heterologous protein. Any reaction in this case was caused by the hydrocarbon portion of the conjugate because in this series no serological reaction occurred between a bovine serum albumin test antigen and the antiserum toward horse serum albumin. The 10-HSA-38 antisera were reactive giving relatively strong tests both with 3,4-benzpyrene and 1,2,5,6-dibenzanthacene haptenes as well as with the homologous hydrocarbon. From the results in this table, it is apparent that the haptenic activity was not completely specific. The 3 hydrocarbons are sufficiently similar that cross reactions occurred in the region of a considerable excess of antigen.

Series IV.—Inasmuch as it was desirable to obtain strong antisera from one conjugate containing other than horse serum albumin, and since bovine serum albumin had been found not to be satisfactory, a conjugate was prepared from human serum albumin.[4] Also, as the best results were obtained with the "10-" series, the conjugate prepared was 1,2-benzanthryl-10-carbamido human serum albumin (10-USA-29).

Albino rabbits were injected with a total of 450 mgm. of the following antigens: HSA (2 rabbits), 10-HSA-16 (4 rabbits), USA (2 rabbits) and 10-USA-29 (4 rabbits). Three intraperitoneal injections of 45 mgm. each per week were given on 3 alternate weeks. Three subcutaneous injections of 10 mgm. each were made per week during the intervening weeks. Two of the 10-USA-29 rabbits were given a total of 250 mgm. of antigen instead of the 450 mgm. given all other rabbits. In view of the observation of Milone, Pesare and Veitch (12), these 2 rabbits and 2 of the 10-HSA-16 rabbits were given 1 cc. of a sterile solution of Wilson's Solubilized Liver Fraction L with each injection of antigen; each rabbit received 700 mgm. of extract. No significant change in antibody content or activity was noted as as result of this extract. The rabbits were exsanguinated by sterile heart puncture 17 to 20 days after

[4] The sample of human serum albumin was supplied by Dr. E. J. Cohn.

TABLE VII: AVERAGE MAXIMUM DEGREE OF PRECIPITATION OF ANTISERA WITH TEST ANTIGENS CONTAINING HOMOLOGOUS AND HETEROLOGOUS PROTEINS, SERIES IV

	Antiserum*			
Antigen	HSA	USA	10-HSA-16	10-USA-29
HSA	+++ (50)†	—	+ (10)	—
USA	—	++ (10)	—	± (3)
BovA	—	—	—	—
10-HSA-16	+++ (100)	—	+++ (5)	
10-USA-29	—	+ (500)		+± (10)

* *See* text.
† The figures in parentheses indicate the number of micrograms of antigen.

the final injection. The sera were obtained and stored in the usual manner. In the serological tests, the results of which are recorded in Table VII, 0.1 cc. of the antiserum was used undiluted with the homologous antigen (0.1 cc.) and diluted by a factor of two for the other test antigens. Undiluted antisera were used in the tests recorded in Table VIII. An examination of these results shows that human serum albumin was not so antigenic as horse serum albumin. Haptenic activity was again demonstrated as shown by the figures in Table VIII. Both the antisera toward 10-HSA-16 and 10-USA-29 showed the strongest haptenic reactions with the test antigens containing the homologous hydrocarbon but they still showed definite reactions with all the heterologous hydrocarbon containing antigens except anthracene, in which case only a questionable reaction occurred.

TABLE VIII: AVERAGE MAXIMUM DEGREE OF PRECIPITATION OF ANTISERA WITH TEST ANTIGENS CONTAINING HETEROLOGOUS PROTEIN AND HOMOLOGOUS OR HETEROLOGOUS HYDROCARBON, SERIES IV

	Antiserum (0.1 cc.)	
Antigen	10-HSA-16	10-USA-29
10-HSA-16		± (50)
10-HSA-38		± (50)
10-BovA-15	tr. 150)*	± (20)
10-BovA-31	± (200)	± (100)
10-USA-29	+ (100)	
BP HSA-19		tr. (250)
BP BovA-19	f. tr. (500)	f. tr. (250)
DbaHSA-21		± (1000)
DbaBov-20	f tr. (50)	tr. (150)
3-HSA-8		tr. (125)
10-Me-3-HSA-16		tr. (100)
3-HSA-9		—

* The figures in parentheses indicate the number of micrograms of antigen.

Since the reactions demonstrating haptenic activity by the precipitin technic were definite but relatively weak (*i.e.* +, ±, tr.), it was considered essential for the proof of haptenic action to conduct the quantitative studies which are presented in the following paper.

SUMMARY

Four series of rabbits were injected with conjugates prepared by the interaction of several serum albumins and other proteins with isocyanates of 1,2-benzanthracene, 1,2,5,6-dibenzanthracene and 3,4-benzpyrene. The resultant antisera were mixed with test conjugates and the degree of precipitation was estimated by the customary visual method. The introduction of the hydrocarbon radical into the protein resulted in the development of haptenic activity and also reduced but did not obliterate the immunological activity of the protein component. The haptenic activity was demonstrated by the reactions which occurred between the test hydrocarbon-protein conjugates and antisera prepared by the injection of conjugates composed of the hydrocarbon prosthetic group attached to a heterologous protein. Haptenic activity was more pronounced in conjugates which contained large numbers of hydrocarbon groups. The most satisfactory conjugates were those prepared from 1,2-benzanthryl-10-isocyanate. Haptenic specificity apparently was not absolute, however. Although antisera with prosthetic group activity directed towards 1,2-benzanthracene reacted most satisfactorily with conjugates containing that hydrocarbon, these sera also reacted with conjugates containing 3,4-benzpyrene, 1,2,5,6-dibenzanthracene and 10-methyl-1,2-benzanthracene.

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Immunological Studies of Hydrocarbon-Protein Conjugates*

II. Quantitative Results**

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Having examined the serological reactions of hydrocarbon-protein conjugates by a customary method (1), it was desirable to obtain quantitative information on the degree of precipitation occurring when various test antigens were added to the antisera. This was accomplished by using larger quantities of antigens and antisera mixed in the proportion which, from the precipitin studies (1), should produce heavy precipitation. The precipitates were removed by centrifugation, washed thoroughly to remove all proteins not involved in the antigen-antibody complex, and analyzed for nitrogen content by the micro-Kjeldahl procedure. This method, which permits accurate comparisons among the test antigens and antisera, has been employed capably by Heidelberger and Kendall (2), Marrack and Smith (3), Pauling, Pressman, Campbell and Ikeda (4), to cite only a few investigateors.

EXPERIMENTAL

Dependent on the degree of precipitation noted in the precipitin tests, 0.5, 1.0 or 2.0 cc. of undiluted antiserum was placed in a 5 cc. centrifuge tube. The actual quantity used was sufficient for the production of an amount of precipitate which could be determined accurately by the micro-Kjeldahl technic. To the antiserum were added a solution of test antigen containing a known amount of antigen and the quantity of 0.85 per cent sodium chloride solution required to bring the total volume in the tube to 4.0 cc. Usually three different amounts of antigen were added to aliquots of each antiserum. To one tube of antiserum was added the amount of antigen calculated to give the maximum amount of precipitate. To the others were added amounts of antigen representing excess antigen in one case and a deficiency of antigen in the other case; the actual amounts were correlated with the extent of the zone of precipitation in the previous precipitin tests. The tubes were incubated at 37° C. for an hour and then placed in the refrigerator overnight. The precipitates were removed by centrifugation, usually at 5° to 10°C., thoroughly mixed and washed three or four times with 5 cc. of 0.85 per cent sodium chloride solution. As shown by other investigators (2-4), no appreciable loss of antigen-antibody complex occurs and the removal of uncombined proteins is complete. The procedure was checked from time to time by running duplicate samples; the discrepancy in results rarely exceeded 3 to 4 per cent. The washed precipitate was suspended in 1 cc. of distilled water and washed into the micro-Kjeldahl digestion flask. Analyses[1] were conducted by a modified Pregl procedure using saturated boric acid solution containing methyl red for the collection of ammonia.

The results in Tables I to III are expressed as mgm. of precipitate obtained per cc. of antiserum when the amounts of antigen (in mgm.), recorded in the headings were added per cc. of antiserum. To avoid using an excessive number of headings in the tables, the amounts of antigen employed are listed within certain ranges, although accurately determined amounts were used in the tests. Different antisera produced by the same antigen were used

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[1] The micro-Kjeldahl analyses were made by E. L. Oginsky, Max Tryon and Edward Walton.

in the tests; each antiserum is identified by a letter to facilitate comparisons of results.

In general, the results of the quantitative tests and the readings of the precipitin tests were in good agreement. In discussing the more important observations, table and line symbols will be used, e.g. II, 12 refers to Table II, line 12.

Various antisera toward any one protein or conjugate showed different degrees of activity. Antisera (a) and (b) towards HSA (I, 1 and 2) produced the same maximum amount of precipitate when tested with HSA but the required amounts of test antigen were different. Also, the amounts of precipitate tapered off differently on both sides of the maximum. Antiserum (c) toward HSA (II, 1) produced less precipitate with HSA and required more antigen than was the case with antiserum (a) or (b). Antiserum (d) toward HSA was exceptional in that it gave much more precipitate with HSA and 10-HSA-38 (II, 2 and 4) than did the other antisera. This particular antiserum behaved abnormally by producing considerable non-specific precipitation with other test antigens, such as BovA and USA, and therefore was not used further in the tests.[2]

In most cases, the unaltered proteins HSA and USA produced stronger antisera than the modified proteins as is shown by their reactions with the homologous antigens (I, 1 and 2; III, 1). In some cases, however, the modified proteins induced the formation of antisera of comparable strength (I, 4 and 13; II, 8, 9 and 10; III, 5). Antisera toward unaltered proteins reacted reasonably well with modified test antigens containing the homologous protein (I, 3; II, 3 and 4; III, 2) showing that the protein component of the conjugates had not completely lost its own serological properties. Similarly, antisera formed by modified proteins gave fair reactions with the unaltered protein homologous to the protein component of the conjugate (I, 7; II, 5, 6, and 7; III, 3 and 4) indicating that the protein component of the conjugate had not been deprived of its own immunizing characteristics. Alterations in the protein component were greater after the introduction of more hydrocarbon groups, e.g., 10-HSA-38 antisera reacted less strongly with homologous conjugates than 10-HSA-16 antisera (I, 8 and 9; II, 8-11).

Table I: Mgm. of Precipitate Produced by Interaction of Various Antisera and Test Antigens

	Antiserum		Antigen	mgm. of Antigen used per cc. of antiserum 10	4-5	2-2.5	.8-1.2	.4-.6	.2-.3	.1-.15	.05	.025
1	HSA	(a)	HSA				0.32			1.80	1.54	1.02
2	HSA	(b)	HSA				0.80		1.81	1.06	0.74	0.35
3	HSA	(a)	BPHSA-19		0.39		0.27	0.41	0.43			
4	BPHSA-4	(a)	BPHSA-19			0.40		0.82	1.36	0.69		
5	BPHSA-4	(b)	BPBovA-19		0.20							
6	BPHSA-4	(b)	DbaBovA-21	0.20								
7	10-HSA-38	(e)	HSA					0.28		0.34		0.46
8	10-HSA-38	(e)	10-HSA-38					0.51		0.49	0.29	
8	10-HSA-38	(a)	10-HSA-38					0.68		0.76	0.55	0.23
10	10-HSA-38	(a)	10-BovA-15			0.34		0.66		0.48		
11	10 HSA-38	(a)	BPBovA-19				0.43					
12	10 HSA-38	(b)	DbaBovA-21	0.25								
13	Dba-HSA-20	(a)	Dba-HSA-20			1.08	1.21	0.97	0.73			
14	Dba-HSA-20	(b)	DbaBovA-21	0.10								

Haptenic activity was shown in all cases by the reactions of antisera toward various conjugates when tested with conjugates containing a heterologous protein component. In these cases the protein component is necessary for antibody formation but the serological reactions result from the influence of the haptenic groups. As was to be expected from the precipitin tests, it was found that the test antigens containing the homologous hydrocarbon produced relatively good reactions with the antisera. Antisera toward 10-HSA and 10-USA showed greater haptenic action (I, 10; II, 12-16; III, 9-13) than antisera toward conjugates containing other hydrocarbons (1, 5 and 14) when tested with conjugates containing the homologous hydrocarbon; this indicates that the most satisfactory haptene was the 1,2-benzanthryl-10 group. The haptenic reactions, however, were not absolutely specific for the homologous hydrocarbon. It was observed that cross reactions also occurred between antisera and "heterologous hydrocarbon-heterologous protein" test antigens although a greater amount of antigen was required (I, 6, 11 and 12; II, 17; III, 14-17). This

[2] Although all tested sera obtained from rabbits prior to injection of the albumins or conjugates have given negative serological results, the serum from this rabbit had not been checked before injection.

TABLE II: MGM. OF PRECIPITATE PRODUCED BY INTERACTION OF VARIOUS TEST ANTIGENS AND ANTISERA TOWARD HSA AND 10-HSA-16

			mgm. of Antigen used per cc. of antiserum									
	Antiserum	Antigen	10	5	25-4	1.2-2.0	.8-1.1	.4-.6	.2-.3	.1-.15	.05	.025
1	HSA	(c) HSA					0.63	1.20	0.95			
2	HSA	(d)* HSA			3.00		7.24	4.10				
3	HSA	(c) 10-HSA-38			0.52	1.35		1.09				
4	HSA	(d)* 10-HSA-38		2.02	4.58	3.90						
5	10-HSA-16	(f) HSA							0.60			
6	"	(h) HSA							0.39	0.31		
7	"	(i) HSA							0.31	0.38	0.25	
8	"	(f) 10-HSA-16				1.18		2.00	1.50			
9	"	(g) 10-HSA-16					1.10			1.18	1.32	1.06
10	"	(h) 10-HSA-16					1.12			1.74	0.72	
11	"	(i) 10-HSA-16					0.68			1.04	1.00	0.82
12	"	(f) 10-BovA-15						0.32	0.35			
13	"	(f) 10-USA-29					0.45	0.58	0.32			
14	"	(g) 10-USA-29					0.30					
15	"	(h) 10-USA-29					0.22	0.34	0.20			
16	"	(i) 10-USA-29				0.31	0.34	0.23				
17	"	(f) BPBovA-19	0.12									

* *See* text.

TABLE III: MGM. OF PRECIPITATE PRODUCED BY INTERACTION OF VARIOUS TEST ANTIGENS AND ANTISERA TOWARD USA AND 10-USA-29

			mgm. of Antigen used per cc. of antiserum								
	Antiserum	Antigen	40	20	8-10	4.5	1.2-2	.62.1	.3-.5	.15-.2	.10
1	USA	(a) USA							0.90	2.04	1.90
2	USA	(a) 10-USA-29		0.27	0.33	0.22					
3	10-USA-29	(a) USA								0.33	
4	"	(d) USA									0.38
5	"	(a) 10-USA-29				0.94		1.42	1.24		
6	"	(b) 10-USA-29					0.76			1.08	0.76
7	"	(c) 10-USA-29					0.20			0.60	0.50
8	"	(d) 10-USA-29				0.51		0.40			
9	"	(a) 10-HSA-16					0.26	0.23			
10	"	(b) 10-HSA-16						0.14			
11	"	(a) 10-HSA-38						0.24			
12	"	(c) 10-HSA-38						0.17	0.20		
13	"	(a) 10-BovA-15						0.19	0.34	0.30	
14	"	(a) BPHSA-19	0.17								
15	"	(a) BPBovA-19				0.19					
16	"	(a) DbaHSA-21	0.22								
17	"	(a) DbaBovA-20					0.12				

observation is of particular significance to the general purpose of the research in that any one conjugate may be expected to produce antisera capable of reacting with similar hydrocarbons as well as with the homologous hydrocarbon. Thus it might not be necessary to have as haptenic group the same hydrocarbon as that used for the production of tumors; a structurally similar one might suffice.

With respect to the number of prosthetic groups required in the conjugates for the demonstration of any appreciable haptenic action, it seems that four 3,4-benzpyrenyl groups were sufficient when the conjugate was used for immunization (I, 5 and 6). Better results, *i.e.*, greater precipitation and at a lower test antigen concentration, were noted generally when greater numbers of groups were present in both the immunizing and test antigens (I, 10 and 11; II, 12-16; III, 13 and 15), although in some cases, antigens with relatively large numbers of prosthetic groups gave rather weak tests (I, 12 and 14; II, 17; III, 14-17). It was noted in the previous paper that the presence in the test antigens of four 3,4-benzpyrenyl groups per molecule of protein was insufficient for haptenic activity. There seems to be little difference in reaction between the 10-HSA-16 and 10-HSA-38 test antigens when used with antiserum toward 10-USA-29 (III, 9-12). It would appear that somewhat better antisera, as far as cross reactions are concerned, were produced by 10-HSA-38 (I, 11 and 12) than by 10-HSA-16 (II, 17), although these antisera behaved similarly when tested with conjugates containing the homologous hydrocarbon (I, 10; II, 12-16).

SUMMARY

The observations of the haptenic action of hydrocarbon-protein conjugates recorded in the preceding paper were confirmed and extended by quantitative studies in which the washed precipitates from the serological tests were analyzed for nitrogen content by the micro-Kjeldahl method. In most cases, the unaltered horse serum albumin and human serum albumin were found to produce somewhat stronger antisera than the hydrocarbon-containing conjugates prepared from these proteins. Haptenic cross reactions were observed among the hydrocarbon prosthetic groups. For the demonstration of haptenic action, a greater number of prosthetic groups was required in the test antigens than in the conjugates used for immunization. The presence of a large number of prosthetic groups in the conjugates seemed more essential for the production of haptenic cross reactions than for homologous haptenic reactions.

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Immunological Studies of Hydrocarbon-Protein Conjugates*

III. Inhibition Reactions

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The results of the preceding papers demonstrated that conjugates, made from proteins and the isocyanates of polycyclic aromatic hydrocarbons, upon injection into rabbits produced antisera capable of reacting serologically with heterologous proteins containing either the homologous or a closely related hydrocarbon. This haptenic action was investigated further with the use of inhibitors composed of the hydrocarbons coupled with either amino acids or peptides

Inhibitors have been used by Landsteiner (8) and many other investigators, to demonstrate haptenic activity. In this test, the antisera toward various types of conjugates are incubated with the haptenic group if water-soluble, or with haptene-amino acid conjugate (7) if the haptene is not soluble in water. Usually, no visible reaction occurs during this stage, but a reaction is shown to have occurred if upon the addition of an appropriate conjugated protein there is no precipitation. This reaction must be controlled, of course, by showing that, without the addition of inhibitors, there is precipitation when the antiserum and conjugated protein are mixed. It is considered that antibodies acquire a configuration different from that of the normal γ-globulin of serum, such alteration being caused by the presence of the antigen at the site of globulin synthesis. The configuration of the antibody is considered to be complementary both to the protein and prosthetic group components of the conjugate. As a result, when such an antiserum is tested (a) with the homologous conjugate or (b) with the protein component, either alone or combined with a heterologous prosthetic group, or (c) with a heterologous protein containing the same prosthetic group, precipitation will occur to a greater or lesser extent. Thus, when the inhibitor is added to an appropriate antiserum, it will combine with the antibody at the regions possessing a pattern complementary to that of the prosthetic group; unless the inhibitor is bivalent (10), there will be no visible reaction. Assuming all experimental conditions to be correct, when a conjugate containing homologous haptene and heterologous protein is added at this stage, no precipitate will form because the antibody configurations at the regions complementary to the haptene group have been occupied by the inhibitor (haptene or haptene-amino acid conjugate). As the rest of the antibody configuration is not complementary to a heterologous protein there will be no serological reaction and, therefore, no precipitation will occur.

From the standpoint of the basic problem of this research, namely the production of antisera capable of preventing the action of carcinogenic hydrocarbons, the inhibition tests should give a somewhat better clue as to the possibility of future success than would the serological tests with antisera and various conjugated proteins. If the antisera are capable of reacting with inhibitors *in vitro*, it is reasonable to expect that antisera *in vivo* can also combine with carcinogenic hydrocarbons and possibly prevent their action. These investigations were in progress at the time when a carcinogenic hydrocarbon by virtue of its own properties was thought to cause tumor formation. If it should be demonstrated that some as yet unknown metabolite is the responsible agent, conjugates containing this metabolite as prosthetic group should be used as immunizing agents.

An unfavorable factor in the projected protective studies is the relatively weak haptenic activity of hydrocarbon prosthetic groups. The inactivation of any carcinogenic hydrocarbon by antibodies stimulated by hydrocarbon-protein conjugates would not be expected to proceed as well as would the inactivation of functional[1] derivatives of hydrocarbons

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[1] The term "functional" means chemically reactive.

(e.g. phenolic or carboxylic acid derivatives) by appropriate antibodies. Counterbalancing this to some extent is the fact that, because of the cross reactions shown by structually similar hydrocarbon as haptenic prosthetic groups, it might not be necessary to employ as haptenic group the same hydrocarbon as used for the production of carcinogenesis. This would permit the use as prosthetic group of some hydrocarbon unlikely to show any carcinogenic activity. A report on the carcinogenicities of several of these conjugates is given by Dunlap and Warren (4). It is of interest to consider the findings of Weigert and Mottram (13, 14) and of Weigert Calcutt and Powell (15) that 3,4-benzpyrene after a few hours contact with tissues produced both aromatic and hydroaromatic functional derivatives prior to the formation of 8-hydroxy-3,4-benzpyrene. Other hydrocarbons probably behave in a somewhat similar manner during the early stages of their metabolism and it is possible that at least some of the prosthetic groups of the hydrocarbon-protein conjugates might be converted to similar derivatives. In such an event, the immunological reactions *in vivo* might involve interactions between functional derivatives of carcinogens and antibodies toward conjugates containing metabolically modified haptenes; if so, antibody-inhibitor reactions *in vivo* should be stronger but more specific in character and the prosthetic group of the conjugate probably would have to be a carcinogenic hydrocarbon. For the successful operation of any protective mechanism of this type, it is obvious that such derivatives undergoing this immunological reaction would have to be elaborated prior to the formation of *the product* (if any) responsible for carcinogenesis and not be subsequently formed inert intermediates in the course of the formation of inactive excretory products.

The main problem in the present study has been to obtain sufficiently water-soluble inhibitors containing the polycycle aromatic hydrocarbon. These are needed to insure the presence in the tests of sufficient haptene to occupy the reactive sites on the antibody molecule and thus prevent any reaction in the subsequent stage of the test. Inhibitors have been prepared by the reaction of the hydrocarbon isocyanates with several amino acids, a dipeptide, a tripeptide and a commercial peptone; in addition, hydrocarbon-polypeptides have been made by the hydrolysis of conjugated proteins.

EXPERIMENTAL

One series of inhibitors was prepared by the reaction of hydrocarbon isocyanates with glycine, ε-amino caproic acid[2] (5) l(+)-glutamic acid, glycyglycine and triglycine (6). Commercial peptone also was conjugated with 1,2-benzanthryl-10-isocyanate in aqueous dioxane solution; the isolated product contained 0.65 mgm. of benzanthryl radical per mgm. of nitrogen. Another series of inhibitors was obtained by the enzymatic degradation[3] of 10-HSA-38, BP-HSA-19, BPBovA-19, Dba-HSA-19, DbaBovA-21, using commercial preparations of pepsin, trypsin pancreatin and erepsin. The course of the hydrolysis was followed by micro-Kjeldahl analyses for total nitrogen and Sorensen titrations using a glass electrode for free amino nitrogen. Corrections were made for the amount of free amino and total nitrogen introduced into the system when the enzymes were added.

A summary of the results of the hydrolyses are given in Table I which shows the percentage of free amino nitrogen (of the total nitrogen) in the starting material and in the hydrolysate at the end of the reaction, as well as the amount of prosthetic group in the isolated product.[4] The hydrolysates were dialyzed against water and saline, freed of insoluble matter by centrifugation and adjusted to pH 3; the precipitate was washed with acetone, dried *in vacuo* and washed with ether to give light brown products which were soluble in water to a moderate extent.

Without attempting any rigid interpretation of the facts presented in Table I, attention may be drawn to the following: (a) the decreased free amino nitrogen content of the conjugates compared with that of the unaltered protein is in reasonable conformity with the amount of hydrocarbon introduced into the protein, (b) hydrolyses proceeded to a stage usually considered to be at the polypeptide level of degradation, (c) the increase in the amount of prosthetic group per mgm. N in the isolated product would tend to indicate that there had been little, if any, hydrolysis of the carbamido linkage joining the hydrocarbon to the protein.

The stock solutions of the digests were diluted

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[2] This compound was made by Edward Walton by the usual method.

[3] Some of the hydrolytes were conducted by Dorothy Campbell Svirbely.

[4] The spectrophotometric determinations were made by Dr. R. N. Jones, Queens University, Kingston, Canada. Because of background absorption, it was not possible to determine the number of hydrocarbon groups in the products of hydrolysis with the high degree of accuracy attained with the hydrocarbon-protein conjugates and the hydrocarbon-amino acid conjugates.

TABLE I: ENZYMATIC DEGRADATION OF HYDROCARBON-PROTEIN CONJUGATES

	Free amino N %	Prosthetic group mgm. per mgm. N
HSA	1.16	0
HSA digest	8.5	0
BovA	1.06	0
BovA digest	8.9	0
10-HSA-26	0.74	0.52
10-HSA-26 digest	7.3	1.4
10-HSA-38	0.54	0.77
10-HSA-38 digest	7.7	1.2
BP Bov A-19	0.66	0.42
BP Bov A-19 digest	6.9	...
Dba HSA-19	0.5	0.52
Dba HSA-19 digest	6.8	1.1
Dba Bov-21	0.6	0.57
Dba Bov-21 digest	5.2	...

serially to 1:16 with 0.85 per cent saline solution. One-tenth of a cubic centimeter of undiluted antiserum was mixed with 0.1 cc. of a digest dilution and incubated at 37° C. for an hour; 0.1 cc. of the optimum concentration of test antigen then was added to the mixture. Control tests were made by using the same quantities of antiserum and antigen and replacing the digest by 0.1 cc. of 0.85 per cent sodium chloride solution. A second series of control tubes contained only antiserum and inhibitor. The tubes were incubated at 37° C., set in the refrigerator overnight and then examined for evidences of reaction.

The hydrocarbon conjugates prepared from amino acids and peptides were dissolved in a solution of N/10 sodium bicarbonate and N/10 sodium carbonate containing a trace of dioxane. The solution was adjusted to pH 7.5 with N/10 hydrochloric acid and any insoluble matter was removed by centrifugation. The stock solutions of these inhibitors were analyzed for nitrogen content and then diluted serially to 1:8. The inhibitors and code are listed in Table II. The tests were made in a similar manner to those in which the digests were used as inhibitors.

TABLE II

Inhibitor	Code
1,2-benzanthryl-10-carbamidoacetoglycine	10-acgly.
" " acetoglycylglycine	10-acglygly.
ε(" ") caproic acid	10-cap.
α(" ")-glutaric acid	10-glut.
glycylglycine	glygly.

A summary of the results of the inhibition tests is given in Table III. The digests were effective in concentrations containing 0.1 to 0.2 mgm. of prosthetic group per 0.1 cc. and the peptide and amino acid conjugates caused inhibition when there was 0.01 to 0.1 mgm. of 1,2-benzanthryl radical per 0.1 cc. of the solution of the inhibitor. The symbols are as follows: O, no inhibition: S, slight inhibition; F, fair inhibition; M, moderate inhibition; P, pronounced inhibition.

Neither the digests of the unmodified proteins HSA and BovA nor the unmodified amino acids and peptides inhibited the reaction between antisera and test antigens. None of the conjugated inhibitors had any effect on reactions between antisera toward unmodified proteins and the homologous protein test antigens. The only inhibitors which by themselves caused any precipitation with the antisera were the digests of Dba-HSA-19 and DbaBovA-21 which were much less soluble than the other inhibitors. Consequently, it was not feasible to assess the inhibitory activity of the 1,2,5,6-dibenzanthryl radical. Digests of 10-HSA-38 and BPBovA-19 showed inhibitory action, the former being the more effective. As was to be expected, the reactions betwen antisera and tests conjugate containing homologous protein (e.g. 10-HSA vs. 10-HSA and 10-USA vs. 10-USA) were inhibited only slightly by any of the inhibitors whereas reactions between antisera and test conjugates containing heterologous protein (e.g. 10-HSA vs. 10-BovA and 10-USA vs. 10-HSA) were inhibited significantly. The 1,2-benzanthryl radical exhibited greater haptenic activity than the 3,4-benzpyrenyl radical in the inhibition tests as well as in the previous serological tests. Of the synthetic inhibitors, the conjugate from ε-aminocaproic acid was the most effective. It exhibited particularly good action when used with antisera and conjugates with heterologous proteins; e.g. acting at a relatively low concentration, it caused pronounced inhibition of the reaction between the test antigen 10-USA and the antiserum toward 10-HSA. The other inhibitors showed a slight to moderate action. It is apparent, therefore, that antisera toward proteins containing hydrocarbon prosthetic groups were able to react with simple derivatives of the hydrocarbons in aqueous solutions.

Based on the results of these studies extensions of the preliminary investigations are now in progress to ascertain if certain of these hydrocarbon-protein conjugates will protect animals effectively against the action of some of the more potent carcinogenic hydrocarbons. Somewhat simliar studies are reported in progress by Lettré, Buchholz and Fernholz (9)

TABLE III: INHIBITION TESTS

Antiserum	Antigen	Digest of 10-HSA-26	Digest of BPBovA-19	Simple inhibitors 10-acgly	10-acglygly	10-cap	10-glut.
10-HSA	10-HSA	F		O	S	S	S
	10-BovA	F	F				
	10-USA			F	M	P	F
	DbaBovA	M	O				
10-USA	10-USA			O	S	S	O
	10-HSA			F	F	P	F
BPHSA	BPBovA	F	F				

and Shabad (12). Theoretically, at least, such an attack is feasible as is shown by the successes in comparable studies by Avery and Goebel (1) with pneumococci, by Clutton, Harington, and Yuill (3) with thyroxin, by Butler, Harington and Yuill (2) with aspirin and by Rodney and Fell (11) with histamine.

SUMMARY

As a further check on the haptenic activity of hydrocarbon-protein conjugates it was demonstrated that antisera toward these conjugates were capable of reacting with enzymatic digests of the conjugates and with simple compounds obtained from the reaction of isocyanates with amino acids and peptides. These results have enabled the establishment of experimental conditions for further investigations of the possibility of protecting animals against hydrocarbon carcinogenesis by the use of some of these conjugates.

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Production of Reticulum Cell Sarcoma and Fibrosarcoma by Methylcholanthrene Adsorbed on Activated Carbon

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Many neoplasms have been produced in experimental animals by introducing by various methods carcinogenic chemicals into different tissues or organs. Relatively few of the tumors induced by these experiments have arisen in the reticuloendothelial system. In 1938 Furth and Furth (1) reported the experimental production of monocytic leukemia in mice by the intrasplenic injection of 1, 2-benzpyrene suspended in lard. In 9 per cent of the mice they found a massive infiltration of the spleen, liver and cervical lymph nodies by large cells resembling histiocytes with some of the characteristics of Hodgkin's disease. In 1939 Mider and Morton (5) painted methylcholanthrene in benzene on the skin of 60 dilute brown mice and produced generalized lymphomatosis, localized mediastinal lymphoma, extramedullary myelopoiesis or reticuloendotheliosis in 48 animals. Only 1 mouse had reticuloendotheliosis with tumor cells in the spleen, liver and lymph modes. In 1940 Roussy, Guérin and Guérin (8) reported the production of reticulum cell sarcoma in the spleen of 2 white rats by implantation of 3,4-benzpyrene within that organ.

The experiment reported in this paper was designed to acertain if an active carcinogenic agent, such as methylcholanthrene, would tend to produce reticulum cell sarcoma if it were localized in the reticuloendothelial system. Since the cells of the reticuloendothelial system are active in phagocytizing particulate matter, it seemed possible that a carçinogenic agent could be prepared which would be selectively taken up by these cells, and could be readily detected in their cytoplasm. With this in mind methylcholanthrene was adsorbed on activated carbon and injected intraperitoneally into mice.

MATERIALS AND METHODS

Sixty milligrams of methylcholanthrene were dissolved in 100 cc. of boiling absolute ethyl alcohol to form a pale yellow solution with a definite blue fluorescense. To this boiling solution 1.2 gm. of activated vegetable carbon were added and the mixture was stirred for 5 minutes. The mixture was allowed to cool. The carbon particles settled rapidly, leaving a clear, colorless supernatant fluid with no fluorescence. The entire mixture was filtered and the carbon was washed with boiling absolute ethyl alcohol. The filtrate and the washings were dried in a weighed evaporating dish. Since no color was observed in the evaporating dish and there was no increase in its weight it was assumed that the methylcholanthrene had been completely adsorbed on the carbon. The resulting methylcholanthrene-carbon complex was suspended in 60 cc. of sterile physiological saline solution so that each cc. contained 1 mgm. of methylcholanthrene and 20 mgm. of carbon.

On January 8, 1944, 30 male white mice, 3 weeks old, were injected intraperitoneally with 1 cc. of the methylcholanthrene-carbon-saline suspension. The mice were from the stock strain used by the Department of Bacteriology at the University of Colorado School of Medicine for the past 8 years. During this time only one spontaneous tumor was noted at autopsy of an estimated thousand mice. The tumor was not examined microscopically. Most of these animals were sacrificed when 9 to 12 months of age. Sixty mice of the same strain were used as controls in this experiment. The controls were kept under identical conditions as to diet and care in the same room, but did not receive the injections given to the test animals.

OBSERVATIONS

At the end of 21 days 2 of the injected animals were sacrificed and examined. On gross inspection, small black masses of carbon were found to be

lightly adherent to the parietal peritoneum, and the mesenteric lymphatic vessels and lymph nodes were distinctly darkened. There was a black film between the liver and spleen and between the liver and diaphragm. The mediastinal lymph nodes were also blackened with carbon. Microscopic examination of paraffin sections, stained by hematoxylin and eosin, showed carbon particles free in the sinuses of the lymph nodes, and within the cytoplasm of the reticuloendothelial cells just beneath the capsule and adjacent to and in the sinuses. The liver contained no carbon in the parenchyma but carbon followed the distribution of the capsule and the fibrous connective tissue septa. The spleen contained no carbon.

At the end of 90 days 3 more animals were sacrificed. The carbon masses were definitely adherent to the peritoneum by what appeared to be a fibrotic reaction. The mesenteric lymph nodes were somewhat enlarged and blackened. The liver was covered with a film of carbon and was adherent to the diaphragm and spleen. Microscopic examination of the black masses adherent to the peritoneum revealed a central nucleus of carbon surrounded by young cellular connective tissue with little inflammatory reaction. Evidence of neoplastic change was not seen. The lymph nodes of all 3 animals contained free carbon in the subcapsular and radial medullary sinuses, and the reticuloendothelial cells in and adjacent to these areas showed hyperplasia and contained carbon in their cytoplasm.

After 132 days, mouse 10 (Table I) had an easily palpable, firm, irregular mass in the left upper quadrant of the abdomen. Two weeks later this animal was sacrificed. Autopsy showed a glistening, firm mass, measuring 15 × 15 ×10 mm., lying between the liver and spleen and adherent to these organs and to the retroperitoneal tissue and intestines (Fig. 1, A). A second tumor of similar size and appearance was attached to the superior surface of the liver and extended through the diaphragm (Fig. 1, B) into the right thoracic cavity where it displaced but did not invade the lung. These tumors were located in areas in which an abundance of carbon had been found in the animals examined at 21 and 90 days. Gross sections of these tumors revealed a central radiating accumulation of carbon pigment surrounded by pale gray, rather firm tissue (Fig. 1). Sections from the area where the tumor invaded the diaphragm (Fig. 2) showed carbon between the liver and diaphragm with a rather abrupt transition from normal diaphragm to tumor, which did not invade the liver. Microscopically the tumor showed a central area of carbon embedded in hyalinized fibrous tissue with an outer zone of anaplastic, spindle cells of various sizes with a few mitotic figures (Fig. 3). This tumor was similar to the methylcholanthrene fibrosarcomas produced in other rodents (4). Five other mice exhibited intra-abdominal tumors of this type in from 139 to 150 days after injection (Table I.).

TABLE I: RESULTS OBSERVED IN 30 MICE INJECTED WITH CARBON-METHYLCHOLANTHRENE, AND IN 60 CONTROLS, OVER A PERIOD OF 180 DAYS

Number of mice	Mouse No.	Tumor noted, days*	Autopsy, days*	Fibrosarcoma	Reticulum cell sarcoma	No tumor noted
2			21			2
3			90			3
1	10	132	146	1		
1	15	139	153	1		
2	22,23	143	157	2		
2	3,26	150	164	2		
1	12	165	170		1	
18			180			18
Total 30				6	1	23
			Controls, not injected			
60			180			60

* Days after injection.

The first reaction to the presence of carbon-carrying methylcholanthrene was the production of numerous normal-appearing fibroblasts as seen in the animals that were sacrificed early. In those animals that developed fibrosarcoma, it would ap-

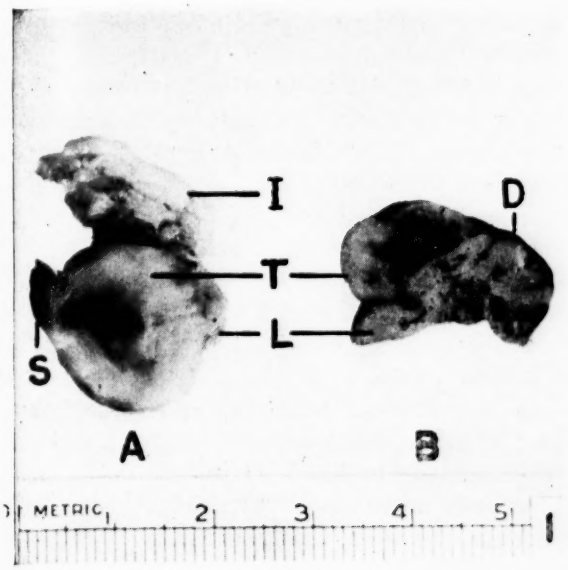
DESCRIPTION OF FIGURES 1 to 4

FIG. 1.—Photograph of 2 fibrosarcomas in mouse 10, 146 days after injection of methylcholanthrene adsorbed on carbon, showing central carbon within the tumors. A.—Tumor (T) between spleen (S) and liver (L) and intestine (I). B.—Tumor attached to superior surface of liver and extending through the diaphragm (D). Mag. × 1.25.

FIG. 2.—Photomicrograph of section from lesion depicted in Fig. 1, B showing fibrosarcoma (T) invading diaphragm (D) but not liver (L). Carbon masses are visible in the tumor and between the liver and diaphragm. Mag. × 30.

FIG. 3.—Photomicrograph of the fibrosarcoma of Fig. 2 showing carbon masses among the tumor cells. Mag. × 400.

FIG. 4.—Photomicrograph of reticulum cell sarcoma in mesenteric lymph node of mouse 12, 170 days after injection of methylcholanthrene adsorbed on carbon. Mag. × 450.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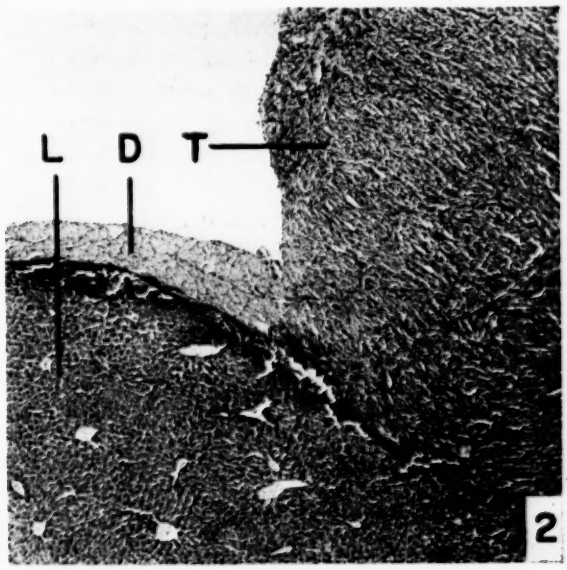
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pear that the continuous action of the carcinogenic agent was responsible for the malignant change. Whether the tumors were the result of the action of methylcholanthrene on the normal-appearing fibroblasts first produced in response to the foreign agent, or whether the fibroblasts were potentially malignant from the beginning could not be definitely decided. However, only 6 of the 30 mice developed fibrosarcomas in the 180 days of this experiment. At any event, fibrosis apparently preceded the formation of fibrosarcomas.

On the 165th day a firm mass was felt in the abdomen of mouse 12 (Table I). This animal was sacrificed on the 170th day. Autopsy revealed a smooth, firm, oval tumor, measuring 15 × 10 × 10 mm., at the root of the mesentery in the usual position of the mesenteric lymph nodes. It had a rather firm gray, homogeneous cut surface with a central mass of carbon extending towards the periphery on one side. Microscopic examination showed a lymph node with no follicles and the architecture completely altered. The central area contained large carbon granules surrounded by a few fibroblasts and collagen fibers. Among them were many poorly defined pleomorphic cells with distinctly outlined hyperchromatic nuclei. Some of these cells were quite large, had homogeneous, pale, acidophilic cytoplasm, and contained large round, oval, sometimes slightly indented, nuclei with 1 to 3 large nucleoli. There were a moderate number of mitotic figures. A few normal-appearing lymphocytes were in this area. Peripherally, the large type of cells became more numerous forming almost a syncytial arrangement (Fig. 4). Some were giant cells with 1 to 3 large nuclei. Carbon pigment was in the cytoplasm of some of the tumor cells. Foot's modification of Bielschowsky's stain showed an abundance of reticulum fibers throughout the tumor, surrounding single or small groups of cells. Van Gieson's stain and Mallory's connective tissue stain showed collagen only in the central area of fibrosis.

The cells making up this tumor were very similar to, but larger than, the hyperplastic, carbon-containing reticuloendothelial cells seen adjacent to the sinusoids in the lymph nodes of mice examined earlier. This tumor was thought to be a reticulum cell sarcoma derived from the reticuloendothelial cells of the lymph node.

Call to military service necessitated the termination of this experiment on the 180th day. All remaining test animals and the 60 control animals were then sacrificed. Autopsy revealed no evidence of neoplasm in any of them.

DISCUSSION

Since the descriptions of malignant reticulum cell tumors of lymph nodes by Goormaghtigh (3), Oberling (6), and Roulet (7), a divergence of opinion has existed as to the exact nature of these neoplasms and there is considerable confusion in their nomenclature. The most popular term in this country is "reticulum cell sarcoma." This has been carelessly broadened to include almost any lymphoid tumor with proliferation of reticular tissue. In 1941 Warren and Picena (9) called attention to the confusion in nomenclature associated with the term "reticulum cell sarcoma" and adopted the restricted criteria of Oberling (6) for diagnosing this tumor. They regarded the reticulum cell sarcoma as the most undifferentiated form of the lymphoid tumors and subscribed to the concept that it originates in the cellular reticulum of the primitive mesenchymal synctium. In 1942, Gall and Mallory (2) reviewed the subject of malignant lymphomas and divided them into seven categories, two of which were grouped together under the heading of "reticulum cell sarcoma." They stated that various authorities regard the type of cell of this tumor "(1) as an immature cell of the lymphocytic series, (2) as a pluripotential cell of variously assumed potentialities of development including the formation of lymphocytes, and of reticulum and collagen, and (3) as a relatively well differentiated cell of the monocyte or clasmatocyte series." Their own observations led them to believe that the reticulum cell sarcoma must be divided into two types: "(1) tumors composed of relatively well differentiated wandering cells with phagocytic propensities resembling monocytes or clasmatocytes, and (2) tumors made up of highly undifferentiated, presumably pluripotential cells" which they chose to call "stem cells."

The tumor that appeared in mouse 12 of our series, apparently fulfilled the criteria of the "undifferentiated or syncytial reticulum cell sarcoma" of Warren and Picena and the "stem cell lymphoma type of reticulum cell sarcoma" of Gall and Mallory.

This experiment was specifically designed to ascertain if methylcholanthrene, when carried within the cells of the reticuloendothelial system, would induce reticulum cell sarcoma. It did so in 1 out of 30 animals. It also produced fibrosarcomas in the peritoneal cavities of 6 other animals of this series. This experiment also demonstrated the ease with which methylcholanthrene can be combined with carbon and incorporated within the cells of the reticuloendothelial system by phagocytic action. The carcinogenic agent was readily tagged by this proce-

dure and made visible in the gross and microscopic examinations. Thus it was possible to observe the development of the tumors in relation to the carbon particles. In the animals sacrificed early, the carbon-methylcholanthrene complex was found in the medullary and cortical lymphatic sinuses and in the reticular cells of these sinuses but not in the cells of the germinal centers of the lymph follicles. The reticular cells near the carbon and those containing carbon were the cells showing hyperplasia, and presumably the reticulum cell sarcoma that developed in mouse 12 arose from these cells. The carbon was equally useful in locating the carcinogen in the fibrosarcomas.

It is possible that a larger number of tumors of both types may have developed had this experiment been continued over a longer period. It is conceivable that more tumors might be produced if the quantity of methylcholanthrene were increased to the maximum amount that could be adsorbed by the carbon. Further experiments have been planned whereby higher concentrations of methylcholanthrene will be used with the carbon, the complex will be given both intraperitoneally and intravenously, and the animals will be observed for longer periods.

SUMMARY

This experiment was designed to ascertain if a known carcinogenic agent would tend to produce reticulum cell sarcoma if it were localized in the cells of the reticuloendothelial system.

Methylcholanthrene was adsorbed on activated carbon to tag the carcinogenic agent visibly and permit it to be phagocytized by cells of the reticuloendothelial system. Thirty white mice were injected intraperitoneally with the carbon-methylcholanthrene complex.

During the 180 days of the experiment, 5 mice were sacrificed within the first 90 days in order to observe the initial reactions. Of those remaining, 6 animals developed typical methylcholanthrene fibrosarcomas within the peritoneal cavity in 132 to 150 days, 1 developed a reticulum cell sarcoma of a mesenteric lymph node in 165 days, and 18 were found free of neoplasms. Omitting the 5 mice sacrificed early, 28 per cent of the injected animals developed tumors. Sixty control mice developed no tumors.

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Desoxycorticosterone Acetate, Mammary Gland Growth, and Carcinogenesis in Mice*

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Whether the carcinogenic action of estrogen is an expression solely of its ability to establish and sustain a mammary gland or whether this hormone has in addition to mammary stimulation an independent carcinogenic property *per se* has been questioned (5). Desoxycorticosterone acetate has been reported to be as effective as estrogen in stimulating the development of mammary glands in young male mice of cancer-susceptible strains (6). If desoxycorticosterone acetate were to sustain mammary stimulation as effectively as estrogen and yet lack carcinogenic potency, it would support the thesis that estrogens are mammary-stimulating and in addition are in a direct sense carcinogenic. It has been reported that sustained treatment of mice with desoxycorticosterone acetate did not lead to mammary tumors (3, 4).

Because in the latter reports (3, 4) no evidence was presented concerning the extent of mammary stimulation, the experiments described here were undertaken to check whether sustained and adequately frequent treatment with desoxycorticosterone acetate[1] would produce verifiable, sustained stimulation of the mammary gland and still not produce tumors.

Male C3H mice from the same stock (colony K. B. DeOme) which developed a high incidence of mammary tumors after treatment with the estrogen triphenylethylene (2) were injected subcutaneously 4 times weekly with 0.05 cc. or 0.1 cc. of 0.5 per cent desoxycorticosterone acetate in sesame oil. Thirty-three animals were injected. Four to six glands were removed from each animal and were prepared as total mounts for microscopic examination to determine the extent of mammary stimulation.

As a control, 10 mice were treated with the estrogen, triphenylethylene. Four times weekly a dose of 0.06 cc. of 2 per cent triphenylethylene (1.2 mgm.) in olive oil was administered. Thus a total of 4.8 mgm. per week in 4 equal doses was given in these experiments. This dosage and frequency of dosage compare favorably with that of 5 mgm. once a week, which in earlier experiments (2) induced tumors after sustained treatment. The minimum dosage and frequency of administration of both desoxycorticosterone acetate and triphenylethylene used in these experiments were those reported (6) to have produced equally marked mammary stimulation.

Table I summarizes the experiments. It may be seen that animals ranging in age from 4 to 34 weeks, intact and castrate, were treated for periods of time ranging from 2 to 23 weeks. In no case did the gland mounts show more than normal mammary development after treatment with desoxycorticosterone acetate. Figs. 1 and 2 are typical of the animals treated with desoxycorticosterone acetate regardless of the length or duration of the treatment. Marked stimulation and growth of the mammary gland resulted from treatment with triphenylethylene after only 8 doses (Figs. 3 and 4).

Treatment was not carried beyond 23 weeks (92 doses) because independent confirmation of negative results came to our attention (1). Mice of strains RIII and 46 were treated twice and thrice weekly respectively with a total of 2 mgm. and 1.5 mgm. per week of desoxycorticosterone in olive oil. A few mice were treated for more than 50 weeks with negative results.

It may be concluded that desoxycorticosterone

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[1] We wish to express our thanks to Dr. Evelyn Anderson of the University of California Medical School for the crystalline desoxycorticosterone acetate.

TABLE I: THE COMPARATIVE EFFECT ON THE MAMMARY GLANDS OF MALE C3H MICE TREATED 4 TIMES WEEKLY WITH DESOXYCORTICOSTERONE AND TRIPHENYLETHYLENE

Initial age, weeks	Hormone	No. of animals Intact	No. of animals Castrate	No. of doses	Total administered, mgm.	Mammary‡ gland development
13	D§	2		36	*	—
17	D	3		52	*	- -
34	D	1		88	*	- -
13	D	2		88	*	- -
13	D	9		92	*	—
6	D	2		8		- -
4	D	2		16		—
9	D	2		20		—
4	D	2		26		—
4	D	1		28		- -
13	D	1		36		—
6	D		6	18		- -
4	T†	5		8		+
13	T†	5		27		+

* For the first 36 doses 0.05 cc. of 0.5% desoxycorticosterone in sesame oil was given per dose; the remaining doses were 0.1 cc. All others receiving desoxycorticosterone were given 0.1 cc. per dose.

† Mice receiving triphenylethylene (T) were given 0.06 cc. of 2% triphenylethylene in olive oil.

‡ The significance of minus and plus used here is demonstrated in Figs. 1 to 4.

§ D refers to desoxycorticosterone acetate.

acetate has slight, if any, stimulating action on the mammary glands of intact or castrate male mice. Its lack of carcinogenic action is therefore irrelevant.

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FIG. 1.—Mammary gland from a C3H mouse showing the small amount of stimulation produced by 36 injections of desoxycorticosterone acetate over a period of 9 weeks beginning when the animal was 4 weeks old. Mag. × 25.

FIG. 2.—Same as Fig 1 except that this animal had received 52 injections of desoxycorticosterone acetate over a period of 13 weeks. Mag. × 7.

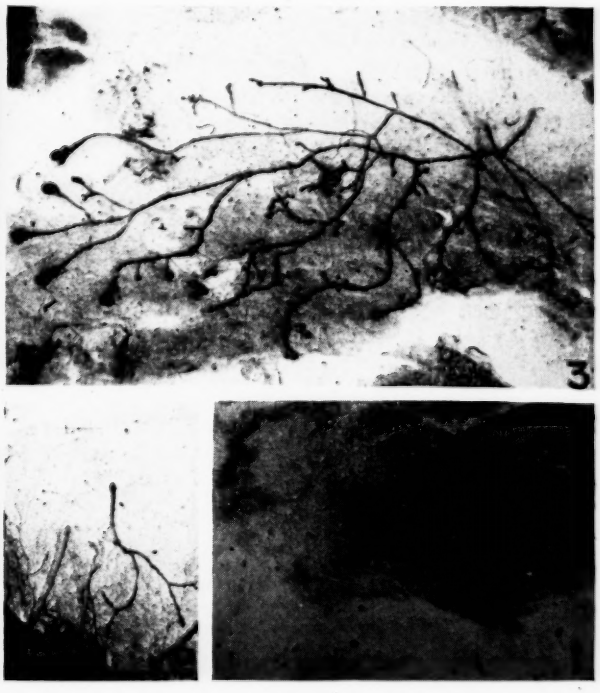

FIG. 3.—A mammary gland from a C3H male mouse showing the extensive proliferation produced by 8 injections of triphenylethylene. The animal was 4 weeks of age at the beginning of the experiment. Mag. × 7.

FIG. 4.—Same as in Fig. 3 except that 27 injections of triphenylethylene were given. Mag. × 7.

Abstracts

Reports of Research

Differences in Rats of Various Strains Shown by Their Rate of Response to Carcinogenic Agents. Lewis, M. R., and King, H. D. [Carnegie Institution of Washington, Baltimore, Md., and Wistar Institute of Anat. and Biol., Philadelphia, Pa.] *Anat. Rec.*, **94**:25-34. 1946.

A total of 267 rats from 15 different strains were injected in the axilla with 0.8 to 4.0 mgm. of 1,2,5,6-dibenzanthracene, 3,4 benzpyrene, or methylcholanthrene, dissolved in olive oil. Regardless of strain, the tumors (sarcomas) developed sooner in males than in females. This is thought to be associated with the more rapid growth rate of the males. In addition there were differences in rate of tumor development among the different strains, ascribed tentatively to differences in genetic constitution. For example, tumors developed much more slowly in gray Norway rats than in Wistar albinos, King inbred albinos, or other strains. Another difference was found between the Wistar albinos and the King inbred albinos. In the King rats sarcomas induced in the subcutaneous tissue were accompanied by pulmonary neoplasms, while in the Wistar rats there were tumors only at the injection site and none in the lungs.—R. B.

Comparison of the Tumours Produced by 2-Acetylamino-Fluorene in Piebald and Wistar Rats. Bielschowsky, F. [Dept. of Path. and Cancer Research Labs; Univ. of Sheffield, Sheffield, England] *Brit. J. Exper. Path.*, **27**:135-139. 1946.

Piebald rats received with their food 4 mgm. of 2-acetylamino-fluorene daily for 25 weeks, and were examined before the 175th day. The differences between the 2 strains may be tabulated as follows:

Tumor	Wistar	Piebald
Epithelioma, ductus acousticus externus	—	much more frequent
Mammary cancer in	Over 60%	1/25*
Tumors of small intestine	7 of 133 ♂ 11 of 166 ♀	over 50%
Carcinoma of colon	14/300*	2/38*

* Number of animals with tumors/number of animals observed.

The histology of the intestinal tumors is described and illustrated. The author does not compare the incidence of liver tumors in the 2 strains and does not state the incidence of intestinal tumors upon untreated piebald rats.—E.L.K.

Production of Metabolic Benzpyrene Derivatives *in Vitro*. Weigert, F., Calcutt, G., and Powell, A. K. [Mt. Vernon Hosp. and Radium Inst., Northwood, Middlesex, England] *Nature, London*, **158**:417. 1946.

Benzpyrene when injected into or painted on mice is converted *in vivo* to metabolic products (X_1 and X_2) distinguished from the hydrocarbon by differences in fluorescence, absorption spectra, and solubility. X_1 is also formed *in vitro* by allowing mouse blood, liver, lung or kidney, or human blood, to act on suspensions of benzpyrene. X_2 is formed *in vitro* by incubating mouse skin with benzpyrene which had been painted on after removal of the skin from the animal. The *in vivo* and *in vitro* products appear to be identical.—I. H.

Carcinogenic Action of Ethyl Urethane on Rats. Jaffé, W. G. [Inst. of Chem. Biol., Caracas, Venezuela] *Cancer Research*, **7**:107-112. 1947.

When albino rats were fed with a diet containing 0.15% of ethyl urethane, 59% developed pulmonary adenomas between 9 and 15 months after the experiment was started. One animal out of 57 showed an incipient hepatoma. Thirty injections of 0.1 mgm. of urethane each caused lung adenomas in 7% of the treated animals while 25% developed hepatomas. An additional intraveneous injection of 2 mgm. of methylcholanthrene did not altar this result. It is concluded that the chronic therapeutic use of urethane in human beings may be harmful.—Author's abstract.

The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents. Jaffé, W. G. [Inst. of Chem. Biol., Caracas, Venezuela] *Cancer Research*, **7**:113-116. 1947.

Six groups of rats were treated with *p*-dimethylamino-azobenzene, methylcholanthrene or ethyl urethane alone, or with a combination of the azo dye and one of the 2 other carcinogens. The number of specific tumors resulting from the combined treatment never exceeded significantly that observed after the treatment with one carcinogen alone. A comparison of the calculated and observed numbers of animals bearing hepatomas and other tumors at the same time showed no significant difference. There was apparently no mutual influence of 2 carcinogenic agents applied at the same time under the experimental conditions employed.—Author's abstract.

Possible Linkage Between the Development of Local Tumors and Pulmonary Adenomas Induced by Methylcholanthrene in Non-Inbred Mice. Jaffé, W. G. [Inst. of Chem. Biol., Caracas, Venezuela] *Cancer Research*, **7**:117-119. 1947.

The relationship between the development of local tumors and pulmonary adenomas in albino mice of a non-inbred strain, which had received a single subcutaneous or intraperitoneal injection of methylcholanthrene, was studied. No significant difference was found between the control and experimental animals regarding the occurrence of both types of these tumors. It is concluded that a non-specific factor, which has been proposed to exist by other authors influencing tumor development, is not necessarily detectable in any strain of mice.—Author's abstract.

For information regarding microfilm copies of articles, abstracts of which appear in *Cancer Research*, application should be made to the Photo-Duplication Section Army Medical Library, 7th Street and Independence Avenue, S.W., Washington 25, D. C.

Production of Tumors in Rats by 2-Aminofluorene and 2-Acetylaminofluorene. Failure of Liver Extract and of Dietary Protein Level to Influence Liver Tumor Production. HARRIS, P. N. [Eli Lilly & Co., Indianapolis, Ind.] *Cancer Research*, **7**:88-94. 1947.

The production of liver tumors in rats by 2-aminofluorene and 2-acetylaminofluorene is not affected by the addition of liver extract to the diet or by variation in casein and riboflavin content of the diet. These compounds produce tumors in a wide variety of locations, the site of tumor development apparently depending upon the genetic constitution as well as upon the sex of the rats employed.—Author's abstracts.

Essais sur la possibilité d'une influence Mammogénique hypophysaire, dans la préparation de la mammelle au développement du carcinome chez la souris. [Studies on the Possibility of an Hypophyseal Mammary Influence on the Preparation of the Mammary Gland for the Development of Carcarcinoma in Mice.] LACASSAGNE, A., and DUBOIS, J. [Inst. of Radium, Pasteur Inst., Paris, France] *Compt. rend. Soc. de biol.*, **135**:49-51. 1941.

Two series of experiments were carried out. (1) Seven young mice of the RIII strain (4 females and 3 males) were gonadectomized when they were 2 months old. Six of these were inoculated subcutaneously with a suspension of whole, fresh pituitary gland from a pregnant mouse. The inoculation was repeated every week, and the animals sacrificed after 30 to 133 days. (2) Fifteen mice, 11 from strain 30 (4 males and 7 females) and 4 females from strain RIII were gonadectomized at various ages (26 to 40 days). One week after the castration, hypophyseal extract from a pregnant cow was injected subcutaneously every 4 days. The animals were sacrificed at various times (35 to 122 days). The conclusions from both series of experiments are that the lactogenic action of the hypophysis of pregnant animals is most marked in ovariectomized young female mice. But even in these cases the canaliculae do not have the special characteristics of proliferation of the acini which are visible in mice of a cancerous strain.—R. J.

Polarographische Untersuchung des Adrenochroms. [Polarographic Investigation of Adrenochrome.] WIESNER, K. [Radiotherap. Inst., Bulovka Hosp., Prague, Czechoslovakia] *Biochem. Ztschr.*, **313**: 48-61. 1942.

Solutions of adrenaline which have been enzymatically oxidized show a polarographic wave which belongs to the oxidation product of adrenaline, *i.e.*, adrenochrome. The reversibility of the adrenochrome-redox system has been polarographically examined and the half-wave potentials at different pH levels corresponding to the redox potentials have been estimated. According to this the redox potential of adrenochrome at pH 7.0 and 20° C. is + 0.044 of a volt against a normal hydrogen electrode. It was observed that the leukostate of adrenochrome was unstable; its decomposition has been followed at different pH values and temperatures. During the reduction with hydrosulfite and hydrazine 2 new cathode waves were observed. In acid solutions the halfwave potential of adrenochrome became negative with the increase of its concentration, although this process seemed to be reversible. An attempt was made to explain this change as a result of dimerization of the oxidized form.—B.S.

Effect of Graded Doses of Thyroxin on Experimental Goiters, Induced by Promizole. HIGGINS, G. M., and JONESON, O. R. [Mayo Foundation, Rochester, Minn.] *Am. J. M. Sc.*, **212**:294-301. 1946.

Promizone (4,2'-diaminophenyl-5'thiazolyl sulfone) appears to exert its goiter-stimulating effect by preventing the synthesis of thyroxin by thyroid cell, thereby permitting an increased elaboration of the thyroid stimulating hormone by the anterior pituitary lobe. The administration of small amounts of thyroxin daily, during the time that the promizole was provided, neutralized the activity of the goitrogen and preserved the thyroid glands in a normal state.—M. T.

Concentration of the Rabbit Papilloma Virus with the Sharples Supercentrifuge. TAYLOR, A. R. [Duke Univ. Sch. Med., Durham, N. C.] *J. Biol. Chem.*, **163**: 283-287. 1946.

This article describes the use of the Sharples supercentrifuge in the preliminary concentration of papilloma virus from large volumes (2 liters) of crude extact. The vacuum type of ultracentrifuge was employed for further concentration and purification.—H.J.C.

The Density and Size of the Rabbit Papilloma Virus. SHARP, D. G., TAYLOR, A. R. and BEARD, J. W. [Duke Univ. Sch. Med., Durham, N. C.] *J. Biol. Chem.*, **163**:289 299. 1946.

Data, calculations and interpretations are recorded and compared with previous work.—H. J. C.

A Study of Three New Duck Variants of the Rous Chicken Sarcoma. DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research*, **7**: 99-102. 1947.

Three new variants in ducks of the Rous sarcoma of chickens were obtained. Because they showed characteristics different from those of the variants previously studied, the strains are described in some detail. The most important difference was that the viruses of the strains here studied, despite becoming adapted to ducks, did not lose their affinity for their original host, the chicken. Also, the tissue affinities of the viruses, the amount of collagen in the tumors, and the reaction of bones of chicks to periosteal and endosteal tumors were different. The virus of one of the variants, strain 14(d)7, was of special interest for the following reasons: (1) because after infecting adult chickens it lost its acquired affinity for ducks and reverted to a chicken tumor type; (2) because it induced malignant tumors in pigeons of all ages; and (3) because it showed an affinity for the central nervous system of young ducks in which system it induced typical lesions.—Author's abstract.

Transmission to Adult Pigeons of Several Variants of the Rous Sarcoma of Chickens. DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] *Cancer Research*, **7**:103-106. 1947.

Suspensions of tumor cells of 2 duck variants of the Rous sarcoma and another duck tumor of uncertain origin injected into the breast of pigeons several months old induced local growths never followed by generalization, whereas tumor cells from another variant induced even in pigeons 12 months old large local growths often followed by generalized metastases and causing death of the host in a few weeks. These tumors have been maintained in 2 or 3 successive passages through pigeons.—Author's abstract.

Human Breast Cancer and the Milk Factor. Macklin, M. T. [Ohio State Univ., Columbus, Ohio.] *Science*, **104**:168-169. 1946.

The author points out certain facts and uncertainties that argue against adopting the suggestion of Hammett (*Science*, **103**:714. 1946.) that women with a family history of breast cancer should not nurse their children. —R. B.

Stromal Malignancy in Mouse-Grown Transplants of Egg-Cultivated Mouse Mammary Carcinoma. Taylor, A., and Carmichael, N. [Univer. of Texas, Biochem. Inst., and Clayton Foundation for Research, Austin, Texas] *Cancer Research*, **7**:78-87. 1947.

A dba mouse mammary carcinoma has been cultivated continuously in eggs for 2 years and 7 months, comprising 80 transplant generations. During that period there has been no observable change in the tumor histology. The stroma of these tumors is made up of chick tissue. Transplants of this egg-grown carcinomatous tissue in mice frequently resulted in the growth of mixed carcinoma-sarcomas. In a series of 186 experiments involving the use of 2,440 mice, it was found that 38% of the tumors from the first transplant generation in mice contained sarcomatous tissue. Subsequent generations of mouse transplants showed stromal malignancy in 52 to 75% of the experiments. The original mammary carcinoma was completely replaced by sarcomatous tissue in 11 of the 186 experiments. The sarcomas produced in this manner were of several cell origins and had varying growth rates and cytological characteristics. Many contained giant cells, nuclear debris and unusual mitotic aberrations. When egg-grown tumor tissue is implanted back into the mouse, the stroma of the resulting tumor is composed of mouse tissue. It is concluded that the induction of malignancy in the normal cells of the stroma is due to contiguity with the cancer cells of the implant and consequent infection with the tumor agent or virus. The process is considered to be essentially the same as that involved in the production of malignant tumors by injection into the mouse of cell-free extracts of materials from tumor-bearing eggs.—Authors' abstract.

Comparison of the Chronic Toxicity of Triethylene Glycol with That of Diethylene Glycol. Fitzhugh, O. G., and Nelson, A. A. [Div. of Pharmacol., Food and Drug Admin., Federal Security Agency, Washington, D. C.] *J. Indus. Hyg. & Toxicol.*, **28**:40-43. 1946.

Diethylene glycol at concentrations of 1, 2, and 4% in the diet of Osborne-Mendel rats (12 per group) for 2 years produced toxic effects. Similar dosages of triethylene glycol produced no toxic effect. Diethylene glycol retarded the growth rate of rats considerably at the high concentration but was statistically significant only during the fast growing period at the 2 lower concentrations. At the dosage levels used neither compound affected food consumption. Diethylene glycol produced lesions in the lower urinary tract related in extent to dosage. Bladder stones of calcium oxalate occurred in all except 1 rat on the 4% concentration, and in lesser numbers in those on the lower concentrations. Bladder tumors occurred in about half of the rats on the 4% and 2% concentration, and in none of the 1% concentration. The tumors were papillary, benign, and intramural. In some cases they showed varying degrees of malignancy. Kidney lesions at the 4% dietary level of diethylene glycol were moderate to marked, at 2% slight to moderate and at 1% slight or absent.

Hepatic lesions paralleled the dosage of diethylene glycol with a moderate amount of damage at 4%, a small amount at 2%, and almost none at 1%. Very little liver damage was visible grossly.

From this and other work the authors conclude that in the ethylene glycol series the chronic oral toxicity to rats decreases with an increase in molecular weight of the glycols.—F. L. H.

The Study of Enzymes in Tissue Sections. Gomori, G. [Univ. of Chicago, Chicago, Ill.] *Am. J. Clin. Path.*, **16**:347-352. 1946.

The author reviews technics and applications of methods for histological demonstration of enzymes by routine laboratory procedures. It is concluded that the importance of these reactions lies not alone in their diagnostic use but also in the fact that they may open up a new avenue of research in experimental pathology.—S. H. D.

Vitamin Content of Mouse Epidermis During Methylcholanthrene Carcinogenesis. I. Biotin, Choline, Inositol, *p*-Aminobenzoic Acid and Pyridoxine. Tatum, E. L., Ritchey, M. G., Cowdry, E. V., and Wicks, L. F. [Barnard Free Skin and Cancer Hosp., Washington Univ. Sch. of Med., St. Louis, Mo.; and Stanford Univ., Santa Clara, Calif.] *J. Biol. Chem.*, **163**:675-682. 1946.

The content of certain vitamins in mouse epidermis following painting with methylcholanthrene in benzene solution was studied by bioassays using mutant strains of Neurospora. The levels of inositol, choline and p-aminobenzoic acid did not change significantly but the biotin content rapidly decreased to about 60% of normal. There was a slight increase in the pyridoxine content after treatment with the carcinogen and with benzene alone.—H. J. C.

Coordination of Cancer Research. Pilcher, K. S. [Cutter Labs., Berkeley, Calif.] *Science*, **104**:167-168. 1946 .

This is a plea for a coordinated, large scale, government-supported attack on the cancer problem.—R. B.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Bone and Bone Marrow

Treatment of Benign Giant-Cell Tumor in the Lower Third of the Femur by Curettage and "Telescoping" the Fragments of Bone. Johnson, R. W., Jr., and Lyford, J., III [Johns Hopkins Hosp., Baltimore, Md.] *J. Bone & Joint Surg.*, **27**:557-561. 1945.

Five cases are presented in which benign giant-cell tumor of the distal end of the femur was treated by obliterating the cavity by telescoping the fragments of bone, after removal of the tumor by curettage and cauterization with phenol. The procedure shortened the leg by 2.5 to 7.5 cm. It permitted full weight-bearing, and good knee function. —M. H. P.

Sarcoma of the Metatarsal Bones. A Brief Review of the Literature, with One Personal Case. THOMAS, I. J. [Cardiff Royal Infirmary, Wales] *Brit. J. Surg.*, 33:191-194. 1945.

Treatment of the various forms of tumor (chondrosarcoma, osteogenic sarcoma, fibrosarcoma) is discussed. The 5 year cure rate is roughly 33%.—E. L. K.

Plasmocytoma of Bone. TENNENT, W. [Selly Oak Hosp., Birmingham Hosp., Birmingham, England] *Brit. J. Surg.*, 32:471-476. 1945.

A case in which primary plasmocytoma remained localized in the right ilium for 4½ years. Subsequently multiple myelomatosis developed and caused death 5 years after the first symptoms. The literature on this subject is summarized.—E. L. K.

Plasma Cell Invasion of Peripheral Blood in Multiple Myeloma. RUBINSTEIN, M. A. [New York, N. Y.] *J. Mt. Sinai Hosp.*, **12**:616-623. 1945.

A case of multiple myeloma is presented in which the diagnosis was first suggested by the presence of plasma cells in the routine blood smear examination. The diagnosis was confirmed by bone marrow studies, and subsequently, massive plasma cell invasion of the peripheral blood occurred. The relationship of plasma cell leukemia and multiple myeloma is discussed.—A. C.

Reticulo-Endothelioma (Spiegler-Fendt Tumour). BAMBER, G. W., FREUDENTHAL, W., and MUENDE, I., *Proc. Roy. Soc. Med.*, **39**:481. 1946.

Description of a case.—E. L. K.

BLOOD VESSELS

Multiple Painful and Painless Glomus Tumors. SLEPYAN, A. H. [U. S. N. R.] *Arch. Dermat. & Syph.*, **50**:179-182. 1944.

A patient presented the unusual picture of two painful glomus tumors associated with 12 painless tumors. The latter lesions contained all the essential features of a glomus tumor. It has been suggested that these tumors are of the nature of telangiectases. Nerve elements were found abundantly in both types of lesions. Surgical excision of the painful tumors led to immediate alleviation of trigger-like pain.—Author's summary.—J. G. K.

Dyschondroplasia with Hemangiomata (Maffucci's Syndrome). Case Report. KRAUSE, C. R. [Med. Corps, U. S. A.] *Am. J. Roentgenol.*, **52**:620-623. 1944.

The case reported is an example of a comparatively mild form of dyschondroplasia with multiple hemangiomas. The patient, a 27 year old soldier, was returned to duty for limited service. No member of his immediate family had had similar tumors. The syndrome is congenital but there is no evidence that it is inherited. —M. H. P.

LEUKEMIA LYMPHOSARCOMA AND HODGKIN'S DISEASE

Acute Monocytic Leukemia. A Case Report. DODGE, H. J., BLACK, W. C., and MUGRAGE, E. R. [Univ. of Colorado Sch. of Med., Denver, Colo.] *Rocky Mt. M. J.*, **41**:177-180. 1944.

This case is added to the literature because it typifies acute monocytic leukemia as to history, course and pathological findings. Monocytic perivascular infiltration of the brain, however, is an uncommon finding.—M. E. H.

Hodgkin's Disease-Incidence and Prognosis. A Statistical Correlation with the Clinicopathologic Picture. BERSACK, S. R. [Vet. Admin. Facility, Hines, Ill.] *Arch. Int. Med.*, **73**:232-237. 1944.

The discussion is based on a study of 225 cases of Hodgkin's disease encountered during an 11 year period. —J. G. K.

Leukemia. A Clinical and Pathologic Study of One Hundred and Twenty-Three Fatal Cases in a Series of 14,000 Necropsies. KIRSCHBAUM, J. D., and PREUSS, F. S. [Woodlawn Hosp., and Cook County Hosp, Chicago, Ill.] *Arch. Int. Med.*, **71**:777-792. 1943.

The cases are classified in 3 ways: (a) according to the clinical course, as acute or chronic leukemia; (b) according to the type cell in the peripheral blood, bone marrow, and tissues, as stem cell leukemia (28 cases), myelogenous leukemia (53 cases), lymphatic leukemia (37 cases), and moncytic leukemia (5 cases); and (c) as leukemic or aleukemic disease.—J. G. K.

Studies in Hodgkin's Syndrome. IV. The Therapeutic Use of Radioactive Phosphorus. HOSTER, H. A., and DOAN, C. A. [Ohio State Univ., Columbus, Ohio] *J. Lab. & Clin. Med.*, **30**:678-683. 1945.

The use of radioactive phosphorus in the treatment of 11 selected patients with Hodgkin's disease in the dosage and under the conditions described has not proved of therapeutic value. Depression of hemocytopoiesis, with special emphasis on thrombocytopenia, has been noted.—Author's summary.—J. G. K.

Ovarian Involvement in Hodgkin's Disease. HELLER, E. L., and PALIN, W. [Univ. of Pittsburgh, and Presbyterian and Woman's Hosps., Pittsburgh, Pa.] *Arch. Path.*, **41**:282-289. 1946.

Report of two cases in which the gross appearance of the lesion was such that it was misdiagnosed as a primary ovarian neoplasm.—J. G. K.

Obscure Lymphosarcoma. BALL, H. A. [San Diego, Calif.] *California & West. Med.*, **64**:82-83. 1946

A case is reported in which the areas of most extensive lymphosarcomatous involvement at autopsy were almost without exception those areas not subjected to radiation previously. The author believes that treatment to the visceral gland-bearing areas lying chiefly along the midline of the thorax and abdomen should be included, even in the absence of symptoms referable to these regions.—V. J. L.

Abdominal Lymphogranulomatosis. CRAVER, L. F., and HERRMANN, J. B. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **55**:165-172. 1946.

In a series of 406 cases of Hodgkin's disease, 13% showed symptoms referable to the gastrointestinal tract and in a small number of these the tract was the primary site of the lesion. Such a primary site represents a distinct entity, and lends itself to surgical treatment. The rest of the cases on the other hand had either secondary gastrointestinal involvement, or extrinsic disease. These conditions usually occurred late in the course of the illness and were found most frequently in patients who had

had the disease for a long time. Symptoms may resemble appendicitis, and may be relieved by x-ray therapy.—E. H. Q.

ADRENAL

Surgical Removal of Adrenal Adenoma with Relief of Cushing Syndrome. WILHELM, S. F., and GROSS, S. [Beth Israel Hosp., New York, N. Y.] *Am. J. M. Sc.*, **207**:196-204. 1944.

Report of a case, with discussion.—J. G. K.

Sex Precocity, Virilism, Adrenal Cortical Tumor. PRATT, J. P., and SCHAEFER, R. L. [Henry Ford Hosp., Detroit, Mich.] *Am. J. Obst. & Gynec.*, **49**:623-633. 1945.

A report of 3 cases of adrenal cortical tumor is given. Clinical manifestations common to all (females) were premature sexual development, virilism, rapid growth, and evidence of a disturbance of adrenal function. The gonads were normal, and there was no evidence of tumors in the region of the third ventricle or pineal gland. No increase in androgens or estrogens was observed. Pseudohemaphroditism was eliminated from the diagnosis because the patients were normal at birth.

The abdominal incision is regarded as preferable in the approach to a suspected adrenal tumor. Both adrenals can be examined as well as the ovaries. Thorough exploration affords valuable information that would be lost if the lumbar route were used.—A. K.

Premature Sexual Precocity in a Young Girl. KARNSKY, K. J. [Jefferson Davis Hosp., and Baylor Med. Sch., Houston, Texas] *J. Clin. Endocrinol.*, **5**:184-188. 1945.

Precocious development of female primary and secondary sexual organs and characters to an extensive degree is described in a 5 year old girl. Titers of urinary gonadotropins, estrogens, and 17-ketosteroids exceeded the range observed in small children. Both ovaries contained many yellow masses which were interpreted as adrenal rests. Similar yellow masses studded the anterior and posterior surfaces of the uterus and broad ligaments and the pelvic peritoneum. Considerable clinical and laboratory data are appended.—J. B. H.

Precocious Sexual and Somatic Development in a Male Infant with a Presacral Teratoma Containing Androgen-Producing Tissue. RHODEN, A. E. [Univ. of Arkansas, Sch. of Med., Little Rock, Ark.] *J. Clin. Endocrinol.*, **4**:185-193. 1944.

A male infant, 9 months of age, was observed to have precocious development of the external genitalia and an exceptionally fast rate of somatic maturation and bodily growth. Upon autopsy the central nervous system, adrenal glands and testes were ascertained to be normal. The thymus was markedly involuted and had great numbers of calcified Hassel bodies. The pathological feature considered to be responsible for this precocity was a presacral teratoma that contained clumps of cells considered to be histologically similar to those of the adrenal cortex or liver, with somewhat less of a resemblance to interstitial cells of the testis. An extract of 55 gm. of the tumor was found to contain an assayable amount of androgenically active material in contrast to renal tissue which was used as a control substance in the assay.—J. B. H.

PANCREAS

Cystadenoma of the Pancreas. A Case Successfully Treated by Surgical Extirpation. BELOFF, J. S., *J. Mt. Sinai Hosp.*, **12**:817-820. 1945.

A case of cystadenoma of the pancreas successfully treated by extirpation is reported in detail. Difficulties in the diagnosis of a pancreatic cyst are illustrated by this case and a discussion of the diagnostic investigation of the disease is presented. It is pointed out that a progressively enlarging mass in the upper abdomen should bring to mind the possibility of a pancreatic cyst. The surgical treatment is extirpation or, if extirpation is not feasible, marsupialization is the procedure of choice.—A. C.

Carcinoma of the Pancreas: A Study of Neoplastic Invasion of Nerves and Its Possible Clinical Signicance. DRAPIEWSKI, J. F. [Mayo Foundation, Rochester, Minn.] *Am. J. Clin. Path.*, **14**:549-556. 1944.

In 84% of 83 cases of carcinoma of the pancreas the pancreatic nerves had been invaded. The incidence of pain was 76% in these 83 cases. It is suggested that neural invasion may be one of the causes of pain, although other mechanisms may have been responsible. Seventy-one per cent of the patients had jaundice. The common bile ducts of 25 of the jaundiced patients were found to be patent. It was postulated, therefore, that a physiologic block resulting from invasion of the wall of the duct and consequent inhibition of the contractive ability of the common duct occurred. The number of cases in which there was metastasis elsewhere than to the neural spaces was determined. No significant conclusions as to the association between metastasis and neural invasion could be drawn. The grades of malignancy of the tumors were determined, and there seemed to be no correlation between the grade of malignancy and the frequency of neural invasion.—J. G. K.

Hypoglycemia Due to Insular Adenoma of Pancreas. ISAACS, H. E. [Beth Israel Hosp., New York, N. Y.] *J. A. M. A.*, **130**:404-406. 1946.

Case report. The rarity of the successful surgical cure of these cases warrants the presentation of this patient who has been free of symptoms for 5 years. Benign adenomas of the islet cells of the pancreas causing the hypoglycemia by hyperinsulinism, should be separated in reports from the malignant tumor of the pancreas with a similar symptom complex but from which the treatment differs radically.—M. E. H.

Convulsive States and Coma in Cases of Islet Cell Adenomas of the Pancreas. HOEFER, P. F. A., GUTTMAN, S. A., and SANDS, I. J. [Coll. of Physicians and Surgeons, Columbia Univ., and Neurological Inst. of New York, New York, N. Y.] *Am. J. Psychiat.*, **102**:486-495. 1946.

The present study is a review of 27 cases of verified islet cell adenoma (benign or malignant) of the pancreas, and includes all cases seen during the past 12 years on the services of the Neurological Institute (14 cases), on the medical (8 cases) and surgical (3 cases) services of Presbyterian Hospital, and at the Harkness Pavilion (2 cases). The tumors were all verified at surgery and by subsequent histological study. The clinical data are arranged in four groups: (1) autonomic-visceral, (2) somatic-neurological, (3) "psychomotor" manifestations,

and (4) seizures and seizure fragments. Glucose tolerance curves in a majority of the cases were diabetic in pattern; flat curves were the exception. The EEG findings were those of patients whose cerebral metabolism is depressed. In addition suggestive "convulsive" patterns such as spike and wave bursts were found in most of the patients even in those with no clinical convulsive seizures.—M. E. H.

Two Cases of Spontaneous Hypoglycemia due to a Tumor of the Islands of Langerhans. Van Beek, C., Haex, A. J. C., and Kooreman, P. J. [State Univ. Leyden, Netherlands] *Acta med. Scandinav.*, **112**:164-187. 1942.

In one case, carcinoma of the islands of Langerhans was found at autopsy. In the other, an adenoma of the islet cells in the head of the pancreas was successfully removed. The diagnosis, treatment, and pathology of spontaneous hypoglycemia are reviewed with 34 references.—M. H. P.

Pineal

Teratoma of Pineal Gland with Choriocarcinoma and Rhabomyosarcoma. Glass, R. L., and Culbertson, C. G. [Indiana Univ. Sch. of Med., Indianapolis, Ind.] *Arch. Path.*, **41**:552-555. 1946.

Report of a case.—J. G. K.

Parotid

Bilateral Adenolymphoma of the Parotid Glands. Ramage, J. S., Binnie, G. G., and McCall, A. J. [North Staffordshire Royal Infirmary, England] *Brit. J. Surg.*, **31**:189-193. 1943.

Only one other record of a bilateral tumor has been found in the literature. The tumor consisted of a peculiar type of columnar epithelium with intracystic papillary processes and an abundant lymphoid stroma.—E. L. K.

Thymus

Surgical Approach for Tumors of the Thymus. Clagett, O. T., and Root, G. T. [Mayo Clin., and Mayo Foundation, Rochester, Minn.] *Surg., Gynec. & Obst.*, **78**:397-401. 1944.

Operative technic is described.—J. G. K.

Thyroid

Malignant Goiter. Ward, R. [Univ. of California Med. Sch., San Francisco, Calif.] *Surgery*, **16**:783-803. 1944.

Almost all malignant goiters are nodular and occur chiefly in the decades between 40 and 70 years. Malignant growths appear in aberrant thyroid tissue at an earlier age. The three cardinal signs of malignant goiter are hoarseness, fixation, and hardness. Surgical therapy is indicated in all nodular goiters before these symptoms are manifest because of the high incidence of malignant change in nodular glands, particularly in men. Statistics from the University of California Hospital through 1943 showed 3,539 nodular goiters in a series of 5,439 thyroids removed. Of the nodular goiters, 168 were malignant (4.8%). Roentgen therapy may be of value postoperatively.—W. A. B.

Poussées goitreuses bénignes et cancer hétérogène grave. [Benign Goiter and Very Malignant Heterogeneous Cancer.] Dargent, M., Guinet, and Bonniot, R. [Anticancer Center, Lyon, France] *Presse méd.*, **54**:305-306. 1946.

Report of 4 cases where the development of a goiter coincided with the appearance of a cancer of high grade malignancy: a nevo-carcinoma, and a carcinoma of the breast, the cervix, and of the thyroid.—C. A.

Parathyroid

Parathyroid Adenoma and Hypertrophy of the Parathyroid Glands. Rogers, H. M. [Mayo Foundation, Rochester, Minn.] *J. A. M. A.*, **130**:22-28. 1946.

Two cases are reported of probable acute hyperparathyroidism not recognized during life. In one patient an adenoma of the parathyroid gland was found at autopsy, and in the other primary hyperplasia of the parathyroid glands was present. Diets high in calcium and phosphorus produced in each instance an exacerbation of symptoms: nausea, vomiting, lethargy, prostration and azotemia.—M. E. H.

Parathyroid Adenoma, with Uremia due to Calcification of the Kidneys. Lober, P., Hertzog, A. J., and Rice, C. O. [Minneapolis General Hosp., St. Barnabas Hosp., and Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Arch. Path.*, **41**:661-667. 1946.

Report of a case.—J. G. K.

Miscellaneous

Commentaires anatomo-cliniques sur le "Ganglion de Troissier," [Anatomical and Clinical Discussion of "Troisier's Ganglion."] Ducuing. M. J. [Toulouse, France] *Bull. Assoc. franç. p. l'etude du cancer*, **30**:2-16. 1942.

The swelling of one or more lmph nodes in the left side of the neck at the angle between the posterior border of the sternocleidomastoid and the superior border of the clavicle is considered by many physicians to be an important sign of gastric cancer. Actually, it may indicate the existence of thoraic or abnominal cancer or even a non-neoplastic disease; in cases of cancer the swelling may be inflammatory but without cancer metastases. The anatomical and clinical features are discussed in the paper. A consideration of the published data and 2,704 cases of cancer seen at the Anticancer Center of Toulouse lead to the conclusion that only in very rare instances is the hypertrophic Troisier's ganglion the first clinical symptom of gastric cancer and it is therefore of little value for early diagnosis.—C. J. L.

Cystic Organoid Teratoma. (Report of a Case.) Smith, E., and Cochrane, W. J. [Dept. of Urol., Royal Victoria Hosp., Montreal, Canada] *Canad. M. A. J.*, **55**:151-152. 1946.

A case of pararenal tumor in a 2 month old infant is reported. Successful removal, with survival 4 years afterwards is of clinical interest. Histological examination revealed tissue derivations of all germ layers in varying degrees and suggests that these tumors originate without the embryonic germinal fold.—M. E. H.

A Fibrolipoma of the Cheek. Noon, C [Norwich, England] *Brit. J. Surg.*, **33**:198. 1945.

A fibrolipoma of the cheek which can be protruded through the mouth by the patient is reported.—E. L. K.

(1) Squamous-Celled Carcinoma of Left Cheek Treated by Operation; (2) Squamous-Celled Carcinoma of Left Tonsil. Patterson, N., *Proc. Roy. Soc. Med.*, **39**:361. 1946.

Description of cases.—E. L. K.

Neoplasm of the Anterior Abdominal Wall with Special Consideration of Desmoid Tumors. Experience with 391 Cases and a Collective Review of the Literature. Pack, G. T., and Ehrlich, H. E. [Memorial Hosp. for Cancer and Allied Diseases, New York, N. Y.] *Surg., Gynec. & Obst., Internat. Abst.*, **79**: 177-197. 1944.

Clinical discussion—J. G. K.

Connective Tissue Nevus. Steiner, K. [New York Post-Graduate Med. Sch. and Hosp., Columbia Univ., New York, N. Y.] *Arch. Dermat. & Syph.*, **50**: 183-190. 1944.

Report of a case with review of the literature.—J. G. K.

Antecedents of Cancer. Rous, P. R., *Lancet*, **2**:98. 1946.

A valuable discussion of carcinogenic factors.—E. L. K.

Avoidable Cancer. Twort, J. M. [Manchester Committee on Cancer, Southwick, Sussex, England] *Lancet*, **2**:215. 1946.

Mineral oil is now being produced which is noncarcinogenic. A mule-spindle oil known commercially as white oil is on the market; this oil is no more carcinogenic than is olive or sperm oil.—E. L. K.

Carcinomas plano cicatricial. [Cicatrizing Carcinomas.] Roffo, A. H., and Taboada, N. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cancer*, **21**:685-698. 1944.

A general discussion, and a tabulation of 45 cases, with photographs.—M. H. P.

Primary Carcinoma of The Extremity. Clarke, H. M. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Surg. Gynec. & Obst.*, **79**:669-672. 1944.

Clinical discussion based on an analysis of 214 cases.—J. G. K.

Carcinoma of the Toe and Metastasis. Report of a Case. Benjamin, A. E. [Minneapolis, Minn.] *Minnesota Med.*, **27**:1032-1033. 1944.

A case report.—R. A. H.

CANCER CONTROL AND PUBLIC HEALTH

Voluntary Funds in Cancer Control. Meiss, E. R. [Connecticut Cancer Society, Inc., Waterbury, Conn.] *Connecticut M. J.*, **10**:648-649. 1946.

Of the money raised 40% will go to the National Cancer Society and 60% will remain in the State. A basic audit of the cancer needs and facilities in the State will be made. Hospitals and communities have been invited to apply for funds for improving local clinics and cancer programs.—M. E. H.

Total War on Cancer. Stebbing, G. F. [Radium Commission, England] *Brit. M. J.*, **2**:77-79. 1946.

A plan for the organization of the diagnosis and treatment of cancer is presented.—E. L. K.

The Tumor Clinic in a Small Hospital. Simon, L. G. [Norwalk General Hosp., Norwalk, Conn.] *Connecticut M. J.*, **10**:765-766. 1946.

The plan of organization for the tumor clinic associated with a small hospital is presented.—M. E. H.

The Chicago Cancer Prevention Clinics. Webster, A., *J. Am. Med. Women's Assoc.*, **1**:12-16. 1946.

The Chicago Cancer Prevention Clinic was opened at the Women and Children's Hospital on May 13, 1943, for the purpose of providing for and encouraging periodic examinations for the early detection of cancerous or precancerous conditions. No treatments are given by the clinic and the patient is sent to her own physician if suspicious lesions are found. A report of its work and some statistics on the types of tumors detected so far are given. —E. B. B.

The Cancer Program in New Jersey. Part I. Diagnostic and Therapeutic Cancer Clinics. [Prepared by the Advisory Committee on Cancer Control, The Med. Society of New Jersey, Trenton, N. J.] *J. M. Soc. New Jersey*, **43**:360-364. 1946.

This is the first in a series of articles on the cancer program in New Jersey. The importance of local cancer clinics is stressed. The organization and operation of tumor clinics of 3 distinct types are outlined. Rules and regulations applicable to cancer clinics are proposed. The cost of establishment, and the equipment necessary is discussed.—M. E. H.

Cancer Prevention Clinics. Macfarlane, C., *J. Am. Med. Women's Assoc.*, **1**:2-3. 1946.

After the first cancer prevention clinic was opened in 1937 in New York by Dr. Elise L'Esperance, the idea of such service has spread rapidly until such clinics now exist in many of the larger cities throughout the country. Their function is to give a complete physical examination, and the patient is referred to his own physician for treatment if evidence of cancer is found. There is but one requirement, that the examinee shall not be under medical treatment at the time of admission to the clinic.

Careful study of the records of 9 clinics reveals that 50% of the persons examined were found to have benign lesions needing surgical or medical care; 25% had precancerous lesions.

The discovery and treatment of cancerous and precancerous conditions is important, but equally important is the educational value of the clinics; for, after receiving a through physical examination, patients leave convinced of the importance of these check-ups and are ready to go out and convince others of their value.—E. B. B.

Book Reviews

ADVANCES IN ENZYMOLOGY AND RELATED SUBJECTS IN BIOCHEMISTRY. Edited by F. F. Nord. Volume VI. New York: Interscience Publishers. 1945, x + 563 pages. Price $6.50.

It is a matter of general agreement that the study of the enzymes involved in a biological process is indispensable for its formulation in biochemical terms. Unfortunately, there is still a wide gap between the knowledge of the enzymes as separate entities and the understanding of their cooperative function in the maintenance of cellular activity. The student of the living cell will, however, be forced increasingly to take account of the available data concerning enzymes. To this purpose, review articles are an esssential aid, especially since the field of enzymology has been characterized, in recent years, by extremely rapid growth and ever-widening scope. The series of volumes entitled "Advances in Enzymology," of which the work under consideration in this review is the sixth, has contributed notably to making available such review articles.

The present volume contains eleven papers. These are: "The Bacterial Amino Acid Decarboxylases," by Ernest F. Gale; "Enzyme Problems in Relation to Chemotherapy, Adaption, Mutations and Immunity," by M. G. Sevag; "Biological Antagonisms between Structurally Related Compounds," by D. W. Woolley; "Adenosinetriphosphatase Properties of Myosin," by V. A. Engelhardt; "States of Altered Metabolism in Disease of Muscle," by Charles L. Hoagland; "Acetyl Phosphate," by Fritz Lipmann; "Microbial Assimilations," by C. E. Clifton; "Chemical Changes in the Harvested Tobacco Leaf. Part I. Chemical and Enzymic Conversions during the Curing Process." by Walter G. Frankenburg; "The Action of the Amylases," by R. H. Hopkins; "The Amylases of Wheat and Their Significance in Milling and Baking Technology," by W. F. Geddes; and "Tocopherol Interrelationships," by K. C. D. Hickman and P. L. Harris.

The recital of the contents of this volume is sufficient to indicate the breadth of scope encompassed in its pages. Only the most catholic student of biochemistry can hope to derive equal stimulus from all the papers. It may be expected, rather, that the readers of *Cancer Research* will choose those review articles which bear more directly on the problems of chemistry and physiology of growth. For this reason, several papers may be selected for special comment.

The paper by Gale provides a masterly account of recent investigations on the enzymic factors involved in the decarboxylation of amino acids by bacteria. This work has already led to the recognition of the important role of pyridoxal phosphate, not only in the amino acid metabolism of bacteria, but of mammalian tissues as well.

Woolley has summarized much of the data on the inhibition of metabolic processes by compounds structurally related to cellular constituents essential for these processes. The discussion of the implication of these fascinating results for the development of a rational pharmacology cannot fail to invite fruitful speculation.

The excellent review by Engelhardt summarizes the knowledge to the end of 1945 concerning the remarkable relationship between myosin and the enzymic hydrolysis of adenosine triphosphate, a key substance in muscular contraction and, more generally, cellular metabolism. This article was written, however, before Cori had reported his success in separating the enzyme from crystalline myosin, and Engelhardt leaves open the question of their identity.

For the student of the biochemistry of disease, the article by Hoagland should have especial interest. The point of view which it brings forth is a significant index of the impact of the development of biochemical knowledge on the study of metabolic disorders. The reviewer must note, with sorrow, the untimely death of Dr. Hoagland in August, 1946.

Of fundamental significance to the study of the energetics of biochemical processes has been the work of Lipmann on "energy-rich" phosphate compounds. One of these substances, acetyl phosphate, has attracted much attention and Lipmann's review of the present status of the subject should be welcomed warmly by all students of enzymology.

JOSEPH S. FRUTON

LES CANCERS PRODUITS PAR DES SUBSTANCES CHIMIQUES EXOGENES. [CANCER PRODUCED BY EXOGENOUS CHEMICAL SUBSTANCES.] Antoine Lacassagne. Paris: Hermann et Cie. 1946, 166 pages.

This volume is welcome testimony of the survival and revival of activity in cancer research in war-torn France. Despite the difficulties and privations imposed by enemy occupation for years, Dr. Lacassagne somehow managed to carry on, and to produce this valuable monograph, written in his usual lucid style.

The book is the fifth in a series entitled "Radiophysiologic Expérimentale: Cancer et Homomes" published under the direction of Dr. Lacassagne. The six chapters deal with the following subjects: arsenic as a carcinogen (Chapter I); carcinogenic azo dyes (Chapter II); carcinogenic polycyclic hydrocarbons. cutaneous cancer produced by skin-painting (Chapter III); carcinogenic polycyclic hydrocarbons, cancer produced by interstitial or intracavitary introduction (Chapter IV); carcinogenic polycyclic hydrocarbons, production of pulmonary and gastric cancer (Chapter V); and carcinogenic polycylic hydrocarbons, general action and production of cancer of the hematopoietic apparatus (Chapter VI). There is a bibliography at the end of each chapter.

The monograph is written from the point of view of the biologist. Another volume, dealing with the chemical

aspects of the subject, is promised (page 86) from the pen of a qualified author. There are seven plates containing the structural formulas of some of the compounds discussed. The biological findings are reviewed critically, and clinical aspects are presented where pertinent. Considerable space is devoted to histological and cytological considerations. There are no photomicrographs or illustrations, probably because of the difficult conditions under which the volume was prepared and published.

The field of chemical carcinogenesis is a complex one with many ramifications. The author makes no pretense of presenting an exhaustive account of the many facets of this subject, but rather gives a resume of selected aspects. The bioliography is not intended to cover all the many contributions, and only those references that are considered most pertinent to the points under discussion are cited. There are some places, however, where a few additional references might make the material more useful. For example, the author states (page 67), quite justifiably, that space does not permit the analysis of the numerous publications on tar cancer which followed the original reports of the Japanese workers; yet mention of Woglom's classic review on tar cancer would provide a critical summary and comprehensive bibliography to those interested.

A valuable feature of this monographs is the inclusion of interesting historical material summarized from publications not readily available in this country.

The reader who may at first be puzzled by the omission of much of the work published in this country since 1940 will probably conclude that many publications, including *Cancer Research* and the *Journal of the National Cancer Institute*, were unavailable to the author as a consequence of wartime conditions in France. It is not to be wondered at that there are some gaps; it is rather an occasion for wonderment that this work should have been done at all, and so well, under such difficult circumstances.

M. J. Shear

COLLOIDS, THEIR PROPERTIES AND APPLICATIONS. A. G. Ward. New York: Interscience Publishers, Inc. 1946, 133 pages, 28 text figures and 4 plates.

This small volume is one of the books issued in Blackie's "Technique" Series, originally published by Blackie & Sons. Ltd., of Glasgow, Scotland. It is presented as "a simple account of the physics and chemistry of colloids, with their many applications in physics, engineering, geology, biology." Drawing its material from advanced reports on the progress in colloid science, this volume is "an attempt to cover the same ground in a more elementary way" for the purpose of serving "as an introduction to the subject for those meeting it for the first time."

The book is divided into three parts as follows: Part I. The Nature of the Colloidal State (5 chapters); Part II. Colloidal Systems (5 chapters): Part III. Colloids in Industry and in Living Matter (7 chapters).

A large amount of information is condensed into relatively short paragraphs, which are written in fairly technical language. As a rapid survey of the state of the knowledge of colloids up to about 1943, this book provides useful orientation, but does not include enough to satisfy those who are seeking detailed knowledge. It gives a helpful outline of the extent of the field and indicates the important applications of the science of colloids.

S. Bayne-Jones

MODERN DEVELOPMENT OF CHEMOTHERAPY. E. Havinga, H. W. Julius, H. Veldstra and K. C. Winkler. New York and Amsterdam: Elsevier Publishing Co., Inc., 1946, 175 pages of text.

One of the impressive features of World War II was the tenacity with which European scientists in occupied countries clung to their research activities. Much of this work was entirely *verboten* and perforce was done in cellars or closets at considerable personal risk. Experimental animals sometimes served the dual purpose of food for thought and food for the investigator. Even while the military occupation was in progress, however, plans were made to publish monographs by the most representative research workers in the Netherlands. With the courageous assistance of the publishing house of Elsevier, a series of these little books was undertaken under the general title "Monographs on the Progress of Research in *Holland* during the War."

The monograph on "Modern Development of Chemotherapy" by E. Havinga, H. W. Julius, H. Veldstra and K. C. Winkler approaches the subject from two main standpoints, *i. e.*, the physico-chemical and the bacteriological. The major portion of the monograph is devoted to the sulphonamides and congeners like *p*-aminobenzoic acid. The mechanism of action is discussed both from the standpoint of bacterial counts under varied circumstances and from the concept of models, e.g., involving electrometric titrations and experiments on mono-molecular surface films. As might be anticipated, much of this work is inconclusive. The synthesis and activity of nearly a hundred chemical derivatives of sulphanilamide are discussed. These compounds are conveniently tabulated and their chemical formulas given in structural form. Ten pages are devoted to pharmacological and clinical studies.

A final chapter is devoted to antibiotics and especially to mycotherapy. This material is devoted almost exclusively to superficial fungus infections.

On the whole, this little book should be regarded as a reader's guide rather than as a treatise. Unfortunately its references contain many foreign items, especially from the Anglo-Saxon literature: so that the Dutch references are greatly diluted. By the time of publication, too, much of the work cited had been well covered in reports from America and elsewhere. Experts in the field of chemotherapy, however, will need access to this little paper-covered monograph and it should be found in every university and pharmaceutical research library. The text is in lucid English and the printer has done a beautiful job in arranging chemical formulas, tables, and graphs.

William T. Salter.